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The Propagation of Filtrable Agents Producing Lymphoid Tumors And Osteopetrosis by Serial Passage in Chickens

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Although numerous experiments have shown that erythro- and myeloid leukosis may be transmitted by a filtrable agent (14), only a few reports have suggested that lymphomatosis may be transmitted by a similar agent.

By the use of filtrates of strain 2, Furth (9) produced what was thought to be a rare type of lymphomatosis. It was characterized by the appearance, in the blood and many organs, of large lymphocytes with occasional formation of lymphoid tumor-like nodules, endothelioma and severe anemia. Pentimalli (16) described a readily transplantable lymphoid tumor strain. Based upon results obtained from filtration, desiccation, and glycerination experiments, he concluded that the strain did not contain a filtrable agent. Olson (15) developed a tumor strain having similar characteristics; however, he did not report on attempts to transmit it by cell-free material. The propagation of 4 lymphoid tumor strains by cell transplantation from cases of naturally occurring visceral lymphomatosis was reported by Burmester and Prickett (3). These strains were similar among themselves and to those of Pentimalli (16) and Olson (15) in that the type of involvement was similar, the incidence of tumor takes was high and the rate of tumor growth was rapid. Brewer and Brownstein (2) have also reported on the rapid transmission of visceral lymphomatosis with suspensions of tumor pulp.

Burmester, Prickett, and Belding (4) in a series of 3 experiments demonstrated the presence of a filtrable agent in the lymphoid tumor originally studied by Olson (15) and obtained at this Laboratory in June, 1942 and since designated here as RPL 12. This filtrable agent failed to induce tumors at the site of inoculation in a short period of time (inocula-containing cells produce tumors at the site of inoculation) but produced within 6 months' time a high incidence of osteopetrosis and lymphomatous tumors of the viscera.

The object of this paper is to report on the propagation of the filtrable agents of the lymphoid tumor strain RPL 12 during 6 serial passages in young chickens and to describe the gross manifestations obtained in the various passages, with different routes of inoculation, and with different types of donors and preparations.

MATERIALS AND METHODS

In the work to be reported here two different types of inoculum were used. The serial passages were made with tumor material or plasma rendered cell-free by centrifugation and filtration. At the same time supplementary inoculations were made in certain instances with tumor cell suspensions or whole blood of the same source as the cell-free preparations.

Preparation of filtrates.—The cell-free extracts of lymphomatous liver tissue were prepared by homogenizing the tumor in 7 parts 0.85 per cent NaCl solution in a Waring Blendor for 20 minutes. Examination of samples by direct and dark-field illumination of wet specimens and fixed smears processed with Wright's stain revealed that after 8 minutes of homogenizing very few intact cells were found, these being mature erythrocytes, but a large number of free nuclei were seen. After 16 minutes no intact cells were found, the number of free nuclei was much less and the innumerable particles seen in the suspension were much smaller than in samples taken after 8 minutes.

For the two inoculations of the second passage Seitz clarifying filters, K1 and K7, were used. The former was more retentive than the latter. Microscopic examination of these filtrates failed to reveal intact cells or free nuclei. Inoculum for the third and fourth passages was prepared by centrifuging the homogenate for 20 minutes at about 3,000 RPM and then filtering through a Seitz S1 pad. The sixth passage was made with material prepared in a similar manner except that 7 parts of

Tyrode's solution at pH 7.1 was used and the centrifuged supernatant was filtered first through a preliminary and then through a regular Mandler candle.

The plasma was obtained from blood which had been withdrawn from the heart of donors into a syringe containing 0.1 volume of heparin solution having a concentration of 0.4 gm. per 100 ml., in 0.85 per cent NaCl solution. It was separated from the cells by centrifugation and then filtered through a sterilizing Seitz S1 pad for passages 1, 3, and 4. A Jenkins-Fisher sterilizing filter was used for passage 5 inoculum.

Forty-eight hour broth cultures of *Serratia marcescens* were used to test all Seitz S1 filters after their use with the extracts and the regular Mandler candle before and after it was used for liver extract. In all cases 1.0 ml. of the filtered broth cultures failed to seed tryptone broth; whereas the unfiltered portion produced typical growth in all tests.

For the extra inoculation of passage 3 the plasma was diluted with equal parts of sterile distilled water. Part of it was then subjected to high-speed centrifugation. It was spun in an angle centrifuge for 20 minutes at 5,000 RPM and the supernatant transferred to six 14-ml. lusteroid tubes and spun at 19,000 RPM (27,000 times the force of gravity). After centrifuging for 2½ hours the contents of each tube was divided into halves, the upper portions were combined and transferred to other lusteroid tubes and the lower portions were similarly combined. The two fractions were again spun for 2½ hours at 19,000 RPM, after which the upper half of the contents of each tube, containing the first upper fraction, was carefully transferred to a serum bottle for inoculation and the lower portion, containing the first lower fraction, was transferred to a second bottle. Gelatinous pellets or sediments were not obtained in either of the two high speed centrifugations.

Preparation of cellular inoculum.—The tumor cells suspensions were prepared by a method already described (3). The whole blood as used for inoculation was not treated after its collection from the donor.

Experimental birds.—The recipient chicks were pedigreed and obtained from matings of an inbred line (line 15) of chickens relatively susceptible to lymphomatosis yet which developed only a few or no cases when maintained under quarantine (17).

The chicks were inoculated at 1 to 3 days of age with 0.25 to 0.5 ml. of the cellular inoculum or 0.5 to 1.0 ml. of the cell-free preparations by the intraperitoneal route except where otherwise indicated. The different inoculation groups of the same passage were reared in the same battery and pens; however, birds of different passages were maintained in separate quarantine pens.

Two groups of non-inoculated control chicks, from the same matings used for the inoculations, were maintained for the first 90 days after hatching in a similar but separate quarantine pen from the inoculated chicks. During the second period of about 90 days, one control group was maintained with birds of the first passage and a second group was kept in the same pen with birds of the extra inoculation of passage 3.

All experimental and control birds were examined after they had died, or were killed to serve as donors, or at the termination of the experiment. All except 2 groups were terminated at 6 months of age. Because of a misunderstanding the birds of the fourth passage were killed at 5 months of age and those of the fifth passage were terminated at 3 months of age. All diagnoses were based on gross alterations observed at necropsy and at periodic clinical examinations. In addition, tissues of all donors were examined microscopically.

PASSAGES AND RESULTS

A summary of the transmission data for the various inoculations and passages is presented in Table I. The results of inoculations representing the first passage of this series have already been presented elsewhere (4). They are included here to facilitate comparison with data of subsequent passages. The inoculum used to initiate this series was plasma obtained from 2 chickens which had received an implant of Strain RPL 12 tumor cells 7 days previously. These donors had large intramuscular tumors at the site of inoculation and diffuse involvement of several visceral organs. The filtered plasma produced tumors in 86 per cent of those inoculated by the intravenous and by the intraperitoneal routes. Most of the birds had tumors in the viscera but many also had osteopetrosis.

Two cases from the first passage were used as donors at 190 and 192 days of age. Both had lymphomatous tumors of the viscera but only one showed evidence of osteopetrosis. Filtrates from both donors produced a high incidence of tumors. The Seitz K7 filtrate of the donor having only visceral tumors produced osteopetrosis in 37 per cent of the recipients, while the Seitz K7 filtrate of the donor with osteopetrosis produced this tumor in 53 per cent of those inoculated. However, osteopetrosis did not occur in a group that had received the Seitz K1 filtrate prepared from the latter donor.

For the third serial passage again 2 donors were

used. Both arose in the group of the second passage that had been inoculated with liver tumor filtrate from a bird showing only visceral tumors. The first donor for the third passage had both osteopetrosis and visceral tumors, and was 148 days of age when sacrificed. Filtered plasma and liver homogenates reproduced a high incidence of both pathologic alterations in the recipients. The second donor showed evidence of only osteopetrosis at 162 days of age. Since the liver was not tumorous only filtered plasma was used. This inoculum produced a high incidence of visceral tumors and osteopetrosis. A difference in the incidence of the two manifestations between the upper and lower high-speed centrifuged fractions of plasma was not obtained.

The fourth serial passage was made with plasma and lymphomatous liver of a 44 day old bird. This donor had been inoculated with filtered plasma of the 148 day old donor of the third passage. By 5 months of age both groups of this passage developed a high incidence of tumors, although there was a marked difference between the two groups in the percentage with visceral tumors. Seventy-seven per cent of the chickens inoculated with filtered liver extract developed lymphoid tumors in the viscera; whereas, only 38 per cent of the plasma-inoculated group developed similar tumors.

The fifth passage was made with plasma of a chicken of the previous passage that had been inoculated with plasma and had developed severe lesions of osteopetrosis but showed no evidence of visceral involvement. The recipients were maintained for an experimental period of only 96 days, during which time 3 of 28 birds inoculated had died with tumors and 3 others were found to have tumors when they were killed on the date of termination.

Birds of the sixth serial passage received a Mandler filtrate prepared from the liver of one of the three birds having tumors at termination of the fifth passage. Eighty-three per cent of the chickens that received this inoculum developed osteopetrosis and 95 per cent (all but one) had tumors in the viscera.

The total tumor incidence for all groups that were inoculated with filtrate and were maintained for 6 months was 81.0 per cent. Most of these (88 per cent) had tumors of the viscera and they died on an average of 137 days after inoculation. About

TABLE I: TRANSMISSION DATA FOR THE SERIAL PASSAGE OF A LYMPHOID TUMOR WITH FILTRATES AND COMPARATIVE INOCULATIONS WITH CELL SUSPENSIONS

Passage No.	Donor used: Days after inoc.	Donor used: Diagnosis: gross	Donor used: Diagnosis: micro.	Inoculum: Source	Inoculum: Filter used	No. chicks inoc.	% with tumors: Bone	% with tumors: Visc.	% with tumors: Nerve	Total % pos.	Average survival visc. cases (days)
1	7	P. V†	P, V, 0	Whole blood	None	4*	0	100	0	100	7
		P, V	P, V	Whole blood	None	4	0	100	0	100	13
				Plasma	Seitz S1	14*	43	57	7	86	132
				Plasma	Seitz S1	14	43	71	0	86	165
				Not inoculated—Controls		14	0	0	0	0	...
2	189	V	V	Liver tumor cells	None	14	7	64	7	72	71
				Liver homogenate	Seitz K7	19	37	68	5	79	147
2 (extra)	187	V, 0	V, 0	Liver tumor cells	None	15	20	87	0	87	84
				Liver homogenate	Seitz K7	15	53	87	7	87	143
				Liver homogenate	Seitz K1	15	0	60	7	60	137
3	145	V, 0	V, 0	Whole blood	None	8	0	63	0	63	43
				Plasma	Seitz S1	20	50	65	5	85	131
				Liver tumor cells	None	10	30	80	0	80	75
				Liver homogenate	Seitz S1	18	33	67	17	78	128
				Not inoculated—Controls		17	0	0	0	0	...
3 (extra)	159	0	0	Whole blood	None	13	15	69	0	77	119
				Plasma	Seitz S1	16	44	69	6	69	135
				Plasma Centrif.	Upper fract.	20	50	70	0	85	136
				Plasma Centrif.	Lower fract.	20	45	65	0	80	131
4‡	43	V, 0	V, 0	Plasma	Seitz S1	16	19	38	12	63	103
				Liver homogenate	Seitz S1	17	12	77	0	77	104
5‡	57	0	0	Plasma	Jenkins	14*	7	29	0	36	...
				Plasma	Jenkins	14	0	7	0	7	...
6	96	V	V	Liver homogenate	Mandler	18	83	95	0	95	140
Total and average percentages for cell-free preparations, exclusive of passages 4 and 5						189	44.4	70.4	4.8	81.0	137.0
				Not inoculated—Controls		31	0	0	0	0	...

*Inoculated by intravenous route, all others intraperitoneal route.
†P = Intramuscular tumors. V = Visceral tumors, O = Osteopetrosis.
‡Passages 4 and 5 were terminated at 5 and 3 months, respectively, all others were terminated at 6 months of age.

one-half (55 per cent) had osteopetrosis and only a few (6 per cent) had neurolymphomatosis.

None of the chickens of the two non-inoculated control groups showed any evidence of tumors or other manifestation of lymphomatosis.

The pathological manifestations obtained with filtrate inoculations of the 6 passages were similar to the visceral tumors and osteopetrosis previously described as the result of inoculations with cell-free preparations of this tumor strain (4). Massive lymphomatous tumors of the viscera occurred in all groups, and osteopetrosis occurred in all except 2 groups inoculated with filtered material. One to 3 cases typical of neurolymphomatosis were observed in 8 of the total of 15 groups inoculated with cell-free material.

Almost half of the positive cases had more than one type of involvement. Of the 153 cases obtained in the filtrate-inoculated birds held for 6 months, 67 had a combination of osteopetrosis and visceral tumors, 66 had visceral tumors without osteopetrosis and only 17 had osteopetrosis without gross evidence of visceral tumors. Of the 9 cases which had nerve involvement all but 3 also had osteopetrosis or visceral tumors.

TABLE II: GROSS INVOLVEMENT OF VISCERAL ORGANS AFTER INOCULATION WITH CELL-FREE MATERIAL

Passage No.	No. of cases	Percentage distribution of Lesions among organs of lymphomatous birds						
		Liver	Spleen	Kidney	Gonad	Heart	Prov.	Perit.
1	11	100	82	73	18	9	9	9
2	28	97	82	53	25	7	4	0
3	51	100	73	39	24	4	4	0
4	15	100	80	80	27	20	0	0
5	5	100	80	80	20	0	0	0
6	17	100	88	76	12	18	0	6
Total	127	99	79	57	22	9	3	2

Gross tumor involvement of the various visceral organs in chickens that died after inoculation with cell-free material is summarized in Table II. Cases showing any visceral involvement almost invariably had lymphomatous livers. Most of them also had spleen and kidney involvement, followed by tumors of the gonad, heart, proventriculus and peritoneum in frequency. Other organs were occasionally involved. No apparent difference in the frequency of involvement of any organ was noted between the various passages, between different routes of inoculation, types of donors used or preparation of inoculum.

Study of tissues from all the donors and a limited number of recipients showed that the microscopic alterations found were uniformly typical of visceral lymphomatosis (8, 11) and osteopetrosis (1, 4, 10).

DISCUSSION

It is apparent that no significant trend or change occurred in the manifestations of this agent during the 6 passages. The type of lesions remained the same, and the incidence of osteopetrosis, visceral tumors, and neural involvement remained at about the same level, although there was a suggestion of an increase in activity since the lowest incidence of osteopetrosis and of visceral tumors occurred in the first and second passages, while the highest incidence occurred in the last or sixth passage. The average age at death was also remarkably similar for the 11 filtrate inoculated groups maintained 6 months.

Data presented in Table I indicate that the rate of tumor growth from transplants of tumors induced by filtrates was much slower than that from transplants of tumors that had been propagated in series with tumor cells, *i.e.*, in serial passage with cellular inoculum. In inoculations of the first 3 passages, groups of chicks were also injected with whole blood or tumor cell suspensions from the same source as the filtrate preparations. Whole blood of birds with 7 day intramuscular transplants produced visceral tumors and death of all birds in an average of 7 (intravenous route) to 13 (intraperitoneal route) days' time with no evidence of osteopetrosis (Table I, passage 1). In contrast, chicks injected with whole blood from lymphomatous and osteopetrotic cases produced by filtrates of the second passage, developed an incidence of 63 and 69 per cent visceral tumors, respectively, and the age at death was prolonged to an average of 43 and 119 days, respectively. Two cases of osteopetrosis occurred in the latter group. Cellular suspensions of lymphomatous livers were also used in inoculation of the second and third passages. The incidence of visceral tumors was high and cases of osteopetrosis appeared in the 3 inoculated groups. The birds died on an average of 84, 71, and 75 days after inoculation.

When birds were inoculated with cell suspensions, prepared from tumors that had been induced by a cellular inoculum, tumors were produced in all birds, the average survival was 7 and 13 days for 2 routes of inoculation (passage 1) and osteopetrosis was not evident;[1] whereas birds in-

[1]This is typical of results obtained at this Laboratory during 55 serial passages of this tumor strain with cellular preparations. All of the 548 birds used in these passages developed tumors, the average survival time was 9.52 days and gross evidence of osteopetrosis was not observed. Microscopic alterations indicative of osteopetrosis were observed in one case (a donor for passage 1 herein described). The lack of appearance of gross bone involvement may have been due to the short survival period, which did not allow sufficient time for grossly visible bone alterations.

oculated with cell suspensions prepared from tumors that had been induced by a cell-free inoculum developed similar tumors, but the incidence was lower, the average survival time was much longer (43 to 119 days) and osteopetrosis was present in all but one group. This longer survival time, which is directly related to the rate of tumor growth and malignancy may be related to the fact that the filtrate-induced tumors used in the transplants took much longer to develop (average of 137 days) than did tumors grown in serial passage with cell transplants (average of 10 days).

A similar difference in results between cell-free preparations of tumors induced by cellular inoculum and cell-free preparations of tumors induced by filtrates was not obtained in these passages. Actually, the filtrate used for the sixth passage produced the highest incidence of tumors and the third passage filtrate group had the lowest age at death (excluding passage 4). However, the differences between these values are small and insignificant. Since the apparent activity of the filtrates remained at about the same level, whereas the apparent malignancy of the tumor cells was much greater in the first than in subsequent passages, one may infer that a positive relation between the malignancy of the tumor cells and virulence of the tumor agent was not obtained in the present experiment.

Tumors in birds that received filtrates were presumably due to a filtrable agent or agents contained therein; however, tumors in birds that were injected with cell suspensions may have been due to direct multiplication of the transplanted cells, or due to an agent within the cells injected or a combination of both. It is significant that except for the first passage (from transplanted tumor) the cellular inocula were no more effective in producing lymphomatous tumors and osteopetrosis than filtrates prepared from the same source.

Although cases of neurolymphomatosis occurred only in inoculated groups the incidence is low and of doubtful significance. Its occurrence may or may not be due to factors other than the inoculum.

During the course of the serial passage inoculations a limited number of transmission variables were tested in a preliminary manner. In the first and fifth passages the intravenous route of inoculation was compared with the intraperitoneal route. The differences obtained were small and not consistent. In the first test the intravenous route caused death in a shorter time but the intraperitoneal route produced the higher incidence of visceral tumors. In the second test a higher incidence in a 3 months' period was obtained with the intravenous route. No difference was obtained in either test in the occurrence of osteopetrosis.

In passages 3 and 4 filtered plasma was compared with the liver filtrate from the same donor. No differences were obtained in the passage 3 test with respect to incidence of osteopetrosis, visceral tumors, or to length of survival time. In passage 4, which was terminated at 5 months, the incidence of visceral tumors in the liver filtrate group was almost twice that in the plasma-inoculated group; however, there was but little difference in the total incidence of tumors. It is thus apparent that an active agent was present in both the lymphomatous liver and in the blood plasma. Differences in the concentration of the active agent could not be estimated because the experimental design does not lend itself to such analyses.

An attempt was made to concentrate the agent in plasma by two centrifugal runs at 19,000 RPM (27,000 times gravity). No difference in transmission was obtained between the upper and lower one-half of the contents of the centrifuge tubes. This result was to be expected since no pellet or other evidence of separation was obtained during this centrifugation. In later experiments, working with muscle tumors, Burmester (5) obtained evidence of sedimentation of the same agent or agents by centrifugation at 19,000 RPM and at 40,000 RPM.

Homogenized lymphomatous liver tissue filtered through a Seitz sterilizing filter in the third passage produced as many tumors as similar material filtered through Seitz clarifying K1 and K7 filters for inoculation in the second passage. Although comparisons of these filters must be made with results obtained with different donors, it would appear that under the conditions of these inoculations the fine filters did not remove much more agent than the coarser ones.

There were variations in the pathological alterations of the donor which were not correlated with similar variation in the recipients. The donors used for passages 2 and 6 had massive lymphomatous involvement of the viscera but no gross or microscopic evidence of osteopetrosis; however, 37 per cent of the birds of passage 2 and 83 per cent of those in passage 6 developed osteopetrosis. This incidence was as high as, or higher than, other passages in which the donor had osteopetrosis.

Donors used for the extra passage 3 and for passage 5 had osteopetrosis without gross or microscopic evidence of lymphomatous involvement of the viscera, yet a high percentage of the recipients developed visceral tumors. Although no attempt was made to separate the two manifesta-

tions during several serial passages, there was no indication of a tendency for one manifestation or the other to become predominant when a donor with only one type was used.

Two explanations may be presented: (a) the two manifestations are due to one and the same agent, and tissue resistance or other similar factors determine the type of involvement obtained, or (b) two separate agents are responsible for the two different manifestations and the alterations obtained are due to the relative activity of each agent. It has already been suggested that osteopetrosis and lymphomatosis may be due to different agents (4). Further evidence of a separate etiology was obtained by differential centrifugation studies (5). The "masked" or "latent" nature of the agent of osteopetrosis was noted by several investigators (1, 7, 10). A similar phenomenon has been demonstrated for Rous tumor virus (13), the Shope papilloma virus (12), and has been suggested for other agents of the avian leukosis complex (7). Thus, there is some evidence suggesting that osteopetrosis and lymphomas of the viscera are produced by different agents, and that either may remain latent in recipients and become overt in subsequent passages.

The results of 8 different inoculations and 2 control groups furnish conclusive evidence that an agent or agents passing through bacteria-retaining filters will induce the formation of osteopetrosis and lymphoid tumors of the viscera. The incidence of grossly visible tumors was high (69 to 95 per cent) in all groups inoculated with the filtrates and maintained for 6 months; whereas no evidence of tumors appeared in two control groups.

Sterilizing Seitz S1 filters were used for the preparation of 7 filtered inocula tested. All filters when tested after filtration of the inocula were found to retain *Serratia marcescens* completely. An 8-pound Mandler candle filter was used for another inoculation. This filter was tested before filtration of the inoculum and again after it was cleaned and resterilized. In all cases, filtrates from 48 hour broth cultures of *Serratia marcescens* were sterile. A Jenkins-Fisher filter[2] was used for the ninth filtrate. This particular filter was not tested for its retention of bacteria; however, 6 filters of the same type and chosen at random were found to completely retain *Serratia marcescens*.

Since all passages after the first were made with filtrates from donors that had received only filtered material, it may be assumed that the agent was propagated in the host as a result of the action of the agent or agents.

[2]Obtained from Fisher Scientific Company, Pittsburgh, Pa.

In working with visceral tumors from cases of naturally occurring lymphomatosis, Burmester and Denington (6) were able to produce a high incidence of lymphomatous tumors with cell-free preparations from 5 of 10 of the original tumors. One of these preparations also produced osteopetrosis. Four tumors were propagated in serial passage with cellular preparations (7). Filtrates prepared from three propagated tumors produced a high incidence of lymphoid tumors within a period of 200 days. The transmission and pathological characteristics of these three strains (RPL 18, 20, and 21) appear to be similar (except for a variation in the incidence of osteopetrosis) to the tumor strain used for experiments reported herein (RPL 12).

SUMMARY AND CONCLUSIONS

1. The filtrable agent or agents inducing osteopetrosis and lymphoid tumors of the viscera were propagated through 6 serial passages in chickens.

2. The incidence of tumors and average survival time were quite uniform for the several filtrate inoculations and passages. An average of 81 per cent of all birds inoculated showed some gross involvement and they died on the average in 137 days. Of the total positive cases 55 per cent had osteopetrosis, 87 per cent had visceral tumors, and 6 per cent had neurolymphomatosis.

3. Results obtained from inoculation of chicks with tumor cell suspensions and filtrates prepared from the same tissue suggest that there was no relation between the malignancy of the tumor cells and the virulence of the agent.

4. After the first passage, filtrates were as effective as cell suspension in producing visceral tumors, and the filtrates invariably produced a higher incidence of osteopetrosis.

5. Filtrates appeared to be about as effective by the intraperitoneal route as by the intravenous route.

6. Filtered plasma of tumor-bearing birds produced about as high an incidence of tumors in recipients as did filtrates of lymphomatous livers.

7. Neurolymphomatosis appeared in 8 of 15 groups inoculated with filtrates but the incidence was not significant.

8. Donors showing only osteopetrosis produced about the same incidence of visceral tumors and osteopetrosis in recipients as donors with only lymphomatous visceral tumors or those with a combination of the two manifestations.

9. Conclusive evidence is presented that this lymphoid tumor contains a propagative agent or agents that will pass through bacteria-retaining

filters and will induce a high incidence of osteopetrosis and visceral tumors in chickens within 6 months' time. The latter tumors have thus far been indistinguishable from the tumors seen in cases of naturally occurring visceral lymphomatosis.

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The Mast Cell Reaction of Mouse Skin To Some Organic Chemicals*

I. Estimation of the Relative Number of Mast Cells in Normal Mouse Skin

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Quantitative determination of the etheral sulphuric acids in the granules of the mast cells in small pieces of tissue would be of considerable value, but unfortunately no micromethods for such extractions and analyses have yet been devised. As matters stand, morphologists have thus to perform a rough estimation of the number of mast cells and their granule content from microscopic slides. The counting of mast cells per tissue unit presents no difficulties *per se*, but the estimation of the amount of metachromatic granules is rather subjective. However, when large differences exist in the number of mast cells and the granule content, such rough methods seem to give conclusive results (6, 7), but minor differences are apt to be overlooked. The methods outlined above are essential for studying the mast cell reaction to different agents. Without entering more closely into the rough quantitative data supplied by earlier authors (1, 2), a more detailed study will be presented in this paper, including a fairly reliable counting technic suitable for experimental work.

Whenever quantitative methods are applied for the demonstration of biological reactions, many questions will arise as to the validity of the control material. For instance, is it possible to determine a "normal" number of mast cells per cu.mm. in the skin of mice? How are they related to age, sex, body weight, nutritional conditions, and so forth? How large are the individual variations in litter mates? *Are the individual variations small enough to permit a reliable "normal value"* in the same region in the skin of mice, which in other respects are comparable?

Furthermore, we recall that some mast cells are rich in granules and will be heavily stained, whereas others are poor in granules and consequently are more difficult to discern. According to Hellström and Holmgren (3) it is desirable to perform the counting in thick sections (about 100 μ) in order to reduce the sources of error, but in such sections granule-poor mast cells are easily overlooked. Thus we are compelled to count these cells in sections of different thickness and the technic must be varied with regard to the material and scope of investigation. Many mast cells in the skin contain only a few small granules, and therefore comparatively thin sections have to be used.

Most skin lesions chemically induced involve inconvenient secondary changes such as edema and inflammatory cell reactions, resulting in an increase in volume of the dermis. A mechanical counting of the number of mast cells per cu. mm. of dermal connective tissue could easily lead to an erroneous decrease in the number of such cells. To avoid the error due to such secondary volume increments, we have correlated the total number of dermal mast cells with the measured length of the epidermis. This is apparently the method of choice under such circumstances.

The methods here reported are specially devised for experimental purposes, *viz.* for further studies on the effect of carcinogenic hydrocarbons, and will deal only with the number of mast cells in the skin of the interscapular region.

Technical Details

A stock of common Swiss albino mice was registered as usual with regard to age, weight and general condition and fed the same mixed diet. Only animals free from abrasions, lice, vermin and fungi were used. With the help of a binocular loupe and small curved scissors the hairs were cut 1 to 2 days before death, and care was taken not to injure the epidermis. Cutting was performed in a rectangular field (1 cm. × 2 cm.) on each side of the spine in the interscapular regions, leaving a small strip of the coat between the fields. Flaps including the whole skin down to the deep external fascia were then excised. The skin flaps, measuring about 0.7 × 1.5 cm., were fastened with thin steel needles to pieces of cork. To

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minimize distortion, faulty stretching and curling of free edges this was done before the last two edges of each flap were cut free. One strip from each cut skin area was then placed for 12 hours in a 4 per cent solution of basic lead acetate (4) and fixed in a mixture of equal parts of formaldehyde solution (14 per cent) and basic lead acetate (8 per cent) for 36 hours. For cytological examination similar flaps were fixed in a solution of formaldehyde, corrosive mercuric chloride and acetic acid. From each flap two sets of paraffin sections were prepared, measuring 4 and 10 μ in thickness respectively. All sections were perpendicular to the skin surface.

All sections of 10 μ were routinely stained in 0.1 per cent toluidine blue solutions in 1 per cent and 30 per cent alcohol (6, 7). The other set of sections was stained for cytological examination. Only sections of 10 μ treated with the basic lead acetate solutions and stained in 0.1 per cent toluidine blue in 30 per cent alcohol solution were accepted for mast cell count.

Methods of Counting

Mast cell counts in thin sections (10 μ) were done by the following methods:

1. One side of a square-ruled ocular micrometer, (described below,) was placed as close as possible to the borderline between epidermis and dermis. Mast cells were then counted separately in that part of the slide corresponding to the upper half of the micrometer net as well as in that corresponding to the lower half. In other words, the number of mast cells was estimated, in the first instance, in the superficial portion of the dermis, and in the second, in the lower dermis and in the hypodermal tissue (Fig. 1). The slide was then moved laterally and a new pair of half-squares were counted as before. Manipulations and counting procedures were repeated 60 times in each case, and the mast cell count thus obtained gave an average value of the number per tissue volume corresponding to the calibrated square rule. The average numbers derived from the right interscapular region of the animals were denoted by *A* and *B* respectively, and refer to the upper and lower halves of the micrometer. Correspondingly, the average numbers of mast cells derived from the left side of the animals have been called *a* and *b*.

Thus,

A and *a* = the *average* number of mast cells (60 observations) in a piece of the superficial dermis measuring 0.01 mm. × 0.0044 sq. mm.

B and *b* = the *average* number of mast cells (60 observations) in a similar piece of deep dermal and hypodermal connective tissue from the left side of the animal.

2. The other method implies that we determine the number of dermal mast cells per 1.0 mm. of epidermal length. This method is designed to avoid the error due to secondary volume increment. Accordingly, a linear calibrated ocular micrometer measuring 1.00 mm. in length is used to apply this standard to the microscopic slides. Thus, 1.00 mm. is plotted with small dots on the slide along the epidermal basement membrane,

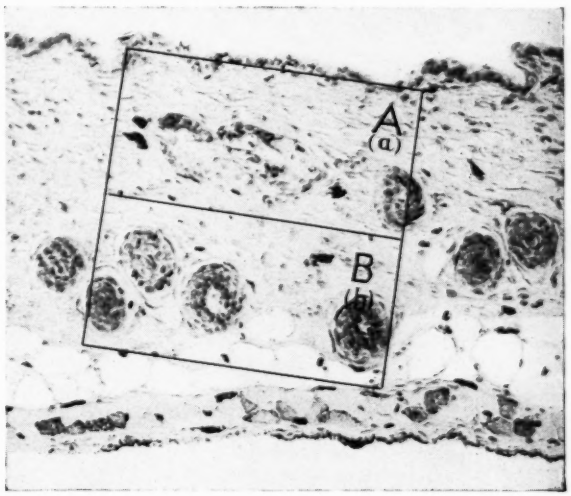

Fig. 1.—Areas for counting of mast cells in normal mouse skin. Mag. × 125.

and then the number of mast cells is counted in the underlying dermal connective tissue. In this way a total of 40 to 50 mm. of skin is examined in sections 10 μ in thickness. To get such a long area of skin, 6 different non-serial sections have usually been used. The average numbers of mast cells in the dermis per mm. of epidermal length was denoted by *C* and *c* corresponding with the designations used above.

C and *c* = the *average* number of *dermal* mast cells per 1.0 mm. of epidermal length, regardless of dermal thickness. (Sections, 10 μ. Number of observations 40 to 50.)

The following standard microscopic equipment was used throughout: Zeiss achromatic objective No. 20, ocular No. 7, and one ocular, square-ruled micrometer net, measuring 0.3 mm. × 0.3 mm. (= 0.09 sq. mm.) with reference to the object.

RESULTS

For the estimation of the number of mast cells, the first method was applied to 10 mice 8 weeks old (Table I). Forty-two additional mice 2 to 3 months old were further examined but for the sake of brevity these results are not recorded in detail. However, it may be mentioned that the

number of mast cells in this material showed the same average value (A and a = 10.3) and also very large individual variations (Table I). Both methods were used in two groups of litter mates (Table II). Only the second method was applied to 2 groups of litter sucklings (Table III).

The figures in Tables I, II and III and those of the additional material mentioned above justify the following conclusions.

cell count obtained in symmetrical skin areas shows remarkable similarity for both sides as manifested by the reported quotients A/a and C/c. The corresponding quotients B/b do not express as great conformity.

Thus, we have found a method suitable for experimental purposes, *viz.* for studying the effect of different agents on the number of dermal mast cells, provided that symmetrical skin areas from

TABLE I: RELATIVE NUMBER OF MAST CELLS IN THE INTERSCAPULAR SKIN OF NORMAL 8 WEEKS OLD SWISS ALBINO MICE

Mouse No.	Age, weeks	Weight, gm.	Number of mast cells: Right side $A \pm E$*	Right side $B \pm E$	Left side $a \pm E$	Left side $b \pm E$	Quotients A/a	Quotients B/b
1†	8	19.5	10.5±0.5	2.7±0.3	9.9±0.6	3.0±0.3	1.06	0.90
2	8	17.	9.0 0.5	2.5 0.3	9.2 0.6	2.6 0.3	0.98	0.96
3	8	14.5	15.4 0.8	4.2 0.4	16.8 1.0	3.9 0.4	0.92	0.93
4	8	17.5	7.1 0.4	3.3 0.3	6.7 0.4	3.0 0.3	1.06	1.10
5	8	20.	9.4 0.6	2.1 0.2	10.4 0.7	2.4 0.2	0.90	0.89
6	8	18.	19.7 0.9	5.1 0.4	21.5 1.0	5.0 0.4	0.92	1.02
7	8	19.5	8.2 0.4	3.2 0.2	8.4 0.5	3.3 0.3	0.98	0.97
8	8	12.5	11.1 0.7	3.5 0.3	10.0 0.6	3.9 0.3	1.11	0.90
9	8	25.	9.2 0.5	2.1 0.2	8.7 0.5	3.0 0.2	1.06	0.70
10	8	13.5	10.3±0.6	3.8±0.3	10.3±0.6	3.2±0.3	1.00	1.19
Average numbers:			11.0	3.25	11.2	3.33		
Standard deviation for quotients:							±0.08	±0.14

*For explanation of symbols see Methods of Counting.
†Mice Nos. 1 to 5 are litter mates.

TABLE II: RELATIVE NUMBER OF MAST CELLS IN THE INTERSCAPULAR SKIN IN A SECOND SERIES OF 6 TO 8 WEEKS OLD SWISS ALBINO MICE

Mouse No.	Age, weeks	Weight, gm.	Number of mast cells: Right side $A \pm E$	Right side $B \pm E$	Right side $C \pm E$	Left side $a \pm E$	Left side $b \pm E$	Left side $c \pm E$	Quotients A/a	Quotients B/b	Quotients C/c
11†	6	19	9.9±0.5	3.8±0.3	40.7±3.5	10.7±0.6	3.9±0.3	42.0±3.5	0.93	0.97	0.97
12	6	16	8.6 0.5	1.8 0.2	28.4 3.0	9.5 0.5	2.2 0.2	27.4 3.1	0.91	0.82	1.03
13	6½	19	7.1 0.4	2.9 0.3	27.2 2.6	7.4 0.4	2.5 0.3	25.0 2.5	0.96	1.16	1.09
14	6½	16	12.8 0.6	3.6 0.3	36.6 3.2	11.9 0.6	3.0 0.3	35.0 3.5	1.08	1.20	1.05
15	7	17	9.6 0.5	3.4 0.3	35.5 3.5	8.4 0.5	3.0 0.3	34.4 3.1	1.14	1.13	1.03
16	7	17	8.0 0.4	2.6 0.3	38.5 4.0	8.7 0.5	2.3 0.2	38.5 3.5	0.92	1.13	1.00
17‡	8	18	10.4 0.6	1.9 0.2	30.3 3.1	11.8 0.6	1.9 0.2	35.1 3.4	0.88	1.00	0.86
18	8	19	10.6 0.6	3.2 0.3	40.2 3.9	10.3 0.6	3.0 0.3	37.5 3.7	1.03	1.07	1.07
19	8½	22	8.4 0.5	1.6 0.2	35.3 3.0	7.5 0.4	1.3 0.2	32.4 3.5	1.12	1.23	1.09
20	8½	18	11.2 0.6	2.5 0.3	43.0 4.2	10.7 0.5	2.0 0.2	38.1 4.0	1.05	1.25	1.13
21	9	18	6.0 0.4	3.6 0.3	28.3 3.0	7.4 0.4	3.4 0.3	30.0 3.2	0.81	1.06	0.94
22	9	18	7.8±0.4	1.8±0.2	30.5±2.8	9.3±0.5	1.9±0.2	34.0±3.1	0.84	0.95	0.90
Average numbers:			A and a = 9.3								
			B and b = 2.6								
			C and c = 34.3								
Standard deviation for quotients:									±0.12	±0.16	±0.09

†Mice Nos. 11 to 16 are litter mates.
‡Mice Nos. 17 to 22 are litter mates.

The *individual variations* of the average numbers of mast cells in the different age groups are very large, both in the dermal (A, a, C, and c) and in the hypodermal (B, and b) material. Even if we could present a "statistically correct" average number of mast cells in mice of different age groups, these figures would be of little or no value to experimental research, because they do not permit any conclusions as to the actual number of mast cells in the individual mouse.

On the other hand, a comparative dermal mast

the same animal always serve as controls. We hardly need repeat that this method so far is applicable only to dorsal skin areas, and is valuable chiefly for studying the strictly dermal mast cells. Furthermore, because of the apparent discrepancy between section thickness and mast cell size, the methods are unsuitable for the estimation of the total numbers of mast cells per tissue unit. If absolute numbers are desired, counting must be performed on thicker sections (3).

The number of mast cells was not found to be

constantly larger in young animals (Table III) than in adults (Table II), and thus the statement by earlier investigators could not be corroborated (1, 2). For lack of additional data this fact will not be discussed (3). In most mice high and low numbers of mast cells in the dermis were found to be consistent with respectively high and low numbers of hypodermal mast cells (Table I and II).

TABLE III: RELATIVE NUMBER OF MAST CELLS IN THE INTERSCAPULAR SKIN OF YOUNG ALBINO MICE COUNTED PER MM. OF EPIDERMIS

Mouse No.	Age, days	Weight, gm.	Number of mast cells Right side $C \pm E$	Left side $c \pm E$	Quotient C/c
23	4	3	19.8 ± 0.4	19.8 ± 0.5	1.00
24	4	3	23.1 ± 0.5	23.4 ± 0.5	0.99
25	4	3.5	14.7 0.8	16.0 0.9	0.91
26	13	6	17.4 0.8	19.4 0.6	1.12
27	13	5	21.3 0.8	18.0 1.5	1.18
28	13	5.5	20.0 ± 1.4	22.2 ± 1.2	0.90
Average numbers:			19.1	19.5	
Standard deviation for quotient:					±0.12

STATISTICS AND ERRORS

The errors inherent in both methods presented above depend upon two different types of error, *vis.* (a) biological and individual variations in the frequency of mast cells, and (b) technical errors due to the methods of preparation. Our primary values are influenced by both groups of errors simultaneously. Due to the fact that our methods imply comparisons between the relative numbers of mast cells in symmetrical skin areas from the same animal, we have avoided the error caused by individual variations. The biological variation in the number of mast cells in the same animal is of course not eliminated. We have to mention the following important technical errors: overstretching of flaps, swelling, shrinkage, faulty section angles, and thickness.

A statistical expression of the distribution of our primary values is obtained by calculating the standard error of the mean for A, B, C, a, b, and c, called E_A, E_B, etc. (Eq. 1). The resulting standard errors for the quotients are calculated, (Eq. 2) and amount to ± 10 per cent for A/a, and ± 15 per cent for C/c. These standard errors do not include systematic technical errors.

If under extremely unfavorable conditions all sources of error go in the same direction, we would get a considerable total error. But fortunately *the actual error usually is much smaller*, as evidenced in Tables I to III. The standard deviation of quotients A/a and C/c amounts to ± 10 per cent (Eq. 3). This expression includes all possible sources of error. Thus, we are justified in stating that these counting methods are fairly applicable to experimental purposes.

Eq. 1
$$E = \pm\sqrt{\frac{\Sigma d^2}{N(N-1)}}$$

Eq. 2
$$E_{A/a} = \pm\frac{A}{a}\sqrt{(E_A)^2 + (E_a)^2}$$

Eq. 3
$$\sigma = \pm\sqrt{\frac{\Sigma d^2}{n-1}}$$

The methods reported above will be used in serial studies on changes in the frequency of visible dermal mast cells induced by different agents. When these standard methods are applied to frequent serial observations resulting in uniform numerical changes, we accept deviations in quotients exceeding 2σ as evidence of true changes in the number of granule-bearing mast cells.

SUMMARY

In the interscapular skin areas on the dorsum of mice of different age groups, the individual variations in the number of dermal and hypodermal mast cells were found to be so large, that it proved impracticable to determine an average standard number. On the other hand, in each individual the numbers of mast cells were found to be about the same in symmetrical skin areas. Using this fact, two simple methods are described for the quantitative assay of the *relative* number of mast cells in thin tissue sections (10 μ). The methods afford ample possibilities for studying the mast cell reactions to different experimental agents, provided that counts for absolute cell numbers per tissue unit are not attempted.

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The Mast Cell Reaction of Mouse Skin To Some Organic Chemicals*

II. The Effect of Common Organic Solvents

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In the course of investigations on the metabolism of ester sulphates and mast cells found in the stroma of human tumors (13-16), it was decided to study some principal stroma reactions separately by experimental means. One line deals with the histochemical reactions occurring during the early phases of skin carcinogenesis. With regard to this topic it was felt that careful reinvestigations on the effects of common organic solvents were badly needed. The following report deals mainly with the skin reactions to single painting with some common solvents. Simultaneous changes in the blood, liver, or urine have not been included.

The application of pure *ether* to the skin causes but very slight epidermal swelling (10). Alcoholic solutions have not been tested. No changes have been observed after painting with pure *acetone* (9, 12). On the other hand, pure *benzene* was found to cause swelling of epidermal cell nuclei and cytoplasm (10), a moderate hyperemia (12), followed by inflammatory response on the part of the dermal connective tissue (10), and subsequent epidermal regeneration, characterized by hyperplasia and hyperkeratosis (10). The skin response to repeated benzene painting has been inadequately studied. Orr (9), however, reported a numerical increase of the number of mast cells in the skin of mice 6 to 8 weeks after painting, "almost to as great an extent as with the carcinogens."

A considerable number of workers have lately shown interest in so-called "detoxication mechanisms" induced by organic chemicals. The protective reactions on the part of the skin constituents will be mentioned below with particular emphasis on the work by Crabtree (1, 2).

Morphology of the Dermal Mast Cells

In new-born mice the mast cells of the corium present a fairly uniform ovoid shape and contain a moderate number of specific granules. During the postnatal development of the skin two separate types of mast cells are differentiated. Small mast cells, irregular mast cells poor in granules are seen in the papillary and superficial part of the reticular layer of corium. In the deeper dermis and in the hypodermal connective tissue the mast cells are larger, rounder, and have more granules. In the first type the granules are very small, often dustlike, but in the second type they are coarser. For further morphological details the reader is referred to comprehensive reviews by Lehner (6), Michels (8), and others.

The metachromatic staining reaction of the granular substance (4) is due to their content of sulphuric acid esters (7). This constitutes a characteristic feature of normal mast cells and enables us to distinguish them from other connective tissue cell elements. Normal tissue mast cells do not present any typical fluorescence in ultraviolet light (3, 11). We have to recall that all morphological descriptions refer to dead mast cells treated by various fixatives and other solvents (benzene, alcohol, etc.) and thus devoid of normal lipids.

Material and Methods

All mice used belong to a common Swiss albino stock of mixed genetic constitution, not subjected to inbreeding. They are resistant to the induction of skin tumors. All animals used were free of skin damages, lice and ringworms. Two days before painting, the hairs were cut by hand as described in the preceding paper (5).

Painting was performed with the following pure solvents:

Alcohol	25% solution in distilled water
Ether	pure anesthetic diethyl ether
Acetone	pure
Benzene	pure acc. Ph. S.

Only the right interscapular region was painted, the symmetrical skin flaps on the left side were used as controls. The animals were killed at daily intervals at the same time, about 10 P.M. One pair of skin strips was cut out for fixation in basic lead acetate, and another pair for fixation in cor-

*Aided by grants from the Caroline Institute, and Consul General Axel Ax:son Johnson, Stockholm.

rosive mercuric chloride solution (5). All technical details concerning the excision of skin flaps, fixation, staining, and preparation of sections were standardized according to methods previously reported (5). Counting of mast cells was undertaken only in isolated (non-serial) sections 10 μ in thickness. Both counting methods described earlier were used (5). In conformity with the foregoing paper we have applied the same statistical methods and demand uniform serial deviations in quotients exceeding $\pm$ 20 per cent as statistical evidence of true changes in the numbers of mast cells (5).

RESULTS

As previously mentioned, only the observations regarding the tissue mast cells will be reported, together with some general tissue changes that are of importance for the interpretation of our findings. Extensive cytological investigations were published by earlier authors.

Alcohol.—A small number of mice 6 weeks old were treated with 12 brush strokes of a 25 per cent solution to the right interscapular region. Skin flaps were taken from both sides 1, 2, 3, 4, 5, and 6 days after the painting. In both the experimental and control skin strips the number of mast cells was found to be of the same magnitude. The quotients showed the following variations:

A/a 0.91 ± 0.08 to 1.14 ± 0.09
B/b 0.94 ± 0.08 to 1.13 ± 0.14
C/c 0.96 ± 0.11 to 1.06 ± 0.14

Standard deviation for quotients was about $\pm$ 10 per cent.

Thus, alcohol painting did not induce conspicuous changes in the number of dermal or hypodermal mast cells within 6 days after painting. Nor were any changes seen in the granular contents.

Ether.—Four full brush strokes of pure ether were applied to the right interscapular region of 15 mice 6 weeks old, and skin flaps, both experimental and control, were examined after 4 hours, daily from the first to the tenth day and on the 12th, 14th, 16th, and 18th days. In all cases the number of mast cells on the experimental side was as great as on the control side, and thus the quotients obtained were about 1. The standard deviation for quotients was similar to that mentioned above. Thus, also in this series there was no effect on the number of mast cells.

No damage to the surface epithelium was observed after the application of ether, and no swelling, thickening or hyperkeratosis could be noted (10).

Acetone.—The effect of pure acetone was studied by painting the right interscapular region of 6 mice 8 weeks old with 3 full brush strokes. Mice were killed 1, 2, 3, 4, 5, and 6 days after painting. The control (left) side was not painted.

Also in this series the same number of mast cells was found in both experimental and control skin flaps. The quotients were about 1.0 and thus, the conclusion was justified that the application of pure acetone did not produce any obvious changes in the number of dermal mast cells.

Apparently, the solvents mentioned above have several characteristics in common. They do not induce conspicuous cell damage, epidermal hyperplasia, noticeable hyperemia or any inflammatory reaction on the part of the dermal connective tissue, at least when used in the reported concentrations and amounts. However, we do not know the amount of resorption of these volatile solvents and their actual effects on the lipid monolayers of the cells. They are of low reactivity and thus do not demand any complicated protective mechanisms on the part of the tissue constituents. Our results indicate that these solvents do not produce any tissue changes involving the granular substance of the mast cells.

Benzene.—The early effects of pure benzene applied to the skin was investigated in two series of mice (Tables I and II, Figs. 1 to 9). Technical details regarding the application of benzene are given under each table. The late effects of single painting with benzene will be reported in a subsequent paper.

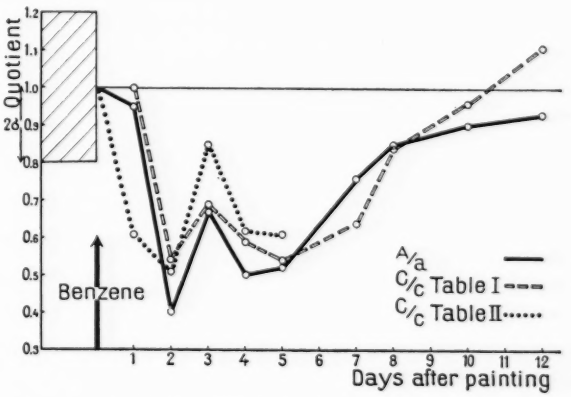


FIG. 1.—Relative number of mast cells in the interscapular skin of mature and young mice after single painting with pure benzene.

Benzene seems to be rapidly absorbed through the intact epidermis. A few minutes after painting many mice show general convulsion of moderate degree. Ten to 15 minutes later they appear, however, to be well again, and remain so until killed.

As to the gross changes a moderate degree of hyperemia was observed in the painted areas during the first 3 days after painting, a fact in accordance with previous statements (12). The microscopic changes were characterized by early swelling of the surface epithelia leading to slight hyperplasia. The dermal edema, described by previous authors, seemed to be moderate and generally more pronounced in younger mice than in mature ones. However, the series are not comparable in this respect because of difference in dosage. Previous statements by Orr (9), and Stowell and Cramer (12) concerning the inflammatory dermal reaction have been verified. Thus, a *slight*, chiefly neutrophilic cell infiltration was found in the dermal connective tissue, but only in places and never throughout the dermis. The cell infiltration was most striking on the second to fifth days after application, then decreased. Neither the edema nor the inflammatory cell infiltration were considerable and did not produce any observable changes in texture of the loose connective tissue. No special attention was paid to the cytological changes in the epidermal cells, or to the epidermal regeneration pattern.

In both series of mice the application of pure benzene apparently induced a considerable decrease in the number of dermal mast cells. This decrease is more than twice the standard deviation for quotients (5), and amounted to about 50 per cent during the second to fifth day after painting (Table I). After the fifth day a slow reappearance of the mast cells was observed. The decrease took place chiefly in the mast cells of the superficial and middle part of the dermis, leaving the hypodermal mast cells comparatively unchanged, as may be seen from the quotient B/b. As mentioned in the preceding paper (5), the second method for counting mast cells excludes the influence of uncontrolable volume increments due to edema and inflammation. When this method was applied to a series of young mice consistent results were obtained (Table II and Fig. 1).

The interpretation is quite clear. We are not justified in believing that the dermal mast cells disappeared from the tissue in question. Instead, they become temporarily invisible. About half of them have lost their specific granules and are no longer stainable with the metachromatic dye. After several days the granular substance gradually resynthesized and thus a number of mast cells reappear in the dermis. These events are amply demonstrated by the observation that the granular content of the superficial mast cells during the first and second days after painting decreases. On the other hand, the reverse observation was established during the fifth to 15th days after painting.

The question, then, is raised as to what happens to the granular substance of the superficial mast

TABLE I: RELATIVE NUMBER OF MAST CELLS IN THE INTERSCAPULAR SKIN OF MATURE MICE AFTER SINGLE PAINTING WITH PURE BENZENE*

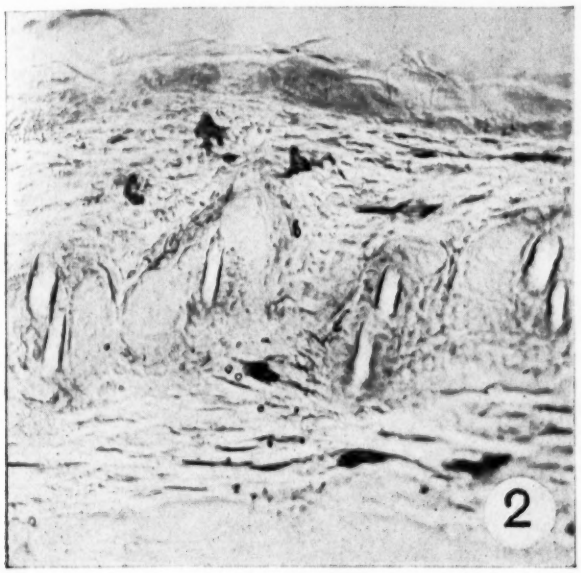
No. days after painting	Weight, gm.	Number of mast cells: Right side $A \pm E$†	Right side $B \pm E$	Right side $C \pm E$	Left side $a \pm E$	Left side $b \pm E$	Left side $c \pm E$	Quotients A/a	Quotients B/b	Quotients C/c
1	18	5.7 ± 0.3	2.1 ± 0.2	24.1 ± 2.3	6.0 ± 0.3	2.2 ± 0.2	24.0 ± 2.5	0.95	0.95	1.00
2	20	3.4 0.3	1.1 0.2	15.0 1.1	8.6 0.5	1.4 0.2	27.7 3.0	0.40	0.79	0.54
3	16	6.2 0.4	2.7 0.3	20.8 1.9	9.3 0.6	2.4 0.3	30.9 2.8	0.67	1.13	0.69
4	20	6.1 0.4	1.8 0.2	16.0 1.7	12.2 0.7	2.1 0.3	27.0 2.9	0.50	0.86	0.59
5	21	4.7 0.4	1.5 0.2	18.8 2.1	9.0 0.5	2.9 0.3	34.9 3.5	0.52	0.52	0.54
7	18	7.4 0.4	2.5 0.2	31.3 2.8	9.8 0.5	3.4 0.3	48.6 4.5	0.76	0.74	0.64
8	21	5.9 0.3	1.8 0.2	28.6 2.6	7.0 0.4	2.0 0.2	34.2 3.1	0.85	0.90	0.84
10	22	9.0 0.5	2.4 0.3	44.3 4.1	10.0 0.5	2.5 0.3	46.0 4.5	0.90	0.96	0.96
12	17	19.8 0.9	5.2 0.4	83.2 7.8	21.3 0.9	6.1 0.4	75.1 7.2	0.93	0.85	1.11
15	22	10.7 0.6	2.7 0.3	49.1 5.1	10.0 0.6	2.6 0.3	54.0 5.1	1.07	1.04	0.91
17	23	9.3 0.5	2.9 0.3	44.9 4.3	9.0 0.5	2.6 0.3	45.3 4.3	1.03	1.12	0.99
22	18	10.0 ± 0.6	3.1 ± 0.3	51.4 ± 4.8	11.0 ± 0.7	2.8 ± 0.3	53.0 ± 5.0	0.91	1.11	0.97
Average numbers for controls:					10.3	2.8	41.7			

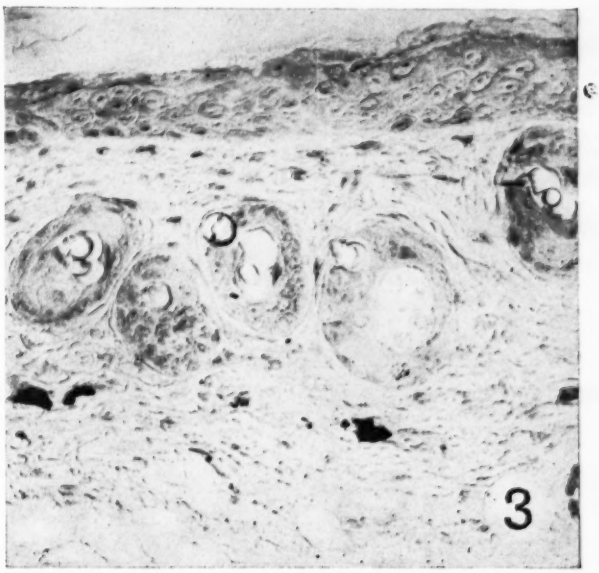
*Mixed Swiss albino mice 8 weeks old were painted with 3 full brush strokes of pure benzene on the right interscapular region of the skin, previously cut. The left side remained untreated and served as control.
†For explanation of symbols, *see* Methods of Counting, in the preceding paper.

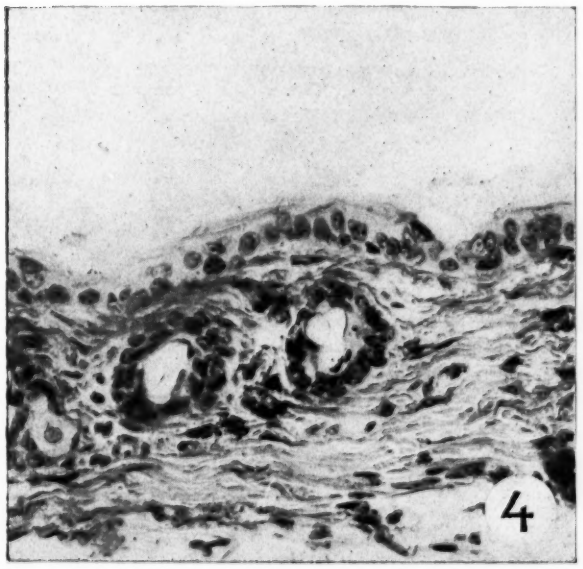
TABLE II: RELATIVE NUMBER OF MAST CELLS IN THE INTERSCAPULAR SKIN OF YOUNG LITTER MATES AFTER SINGLE PAINTING WITH PURE BENZENE*

Age, days	No. days after painting	Weight, gm.	Number of Mast Cells: Right side $C \pm E$†	Left side $c \pm E$	Quotients $C/c \pm E$
14	1	4.5	23.6 ± 2.9	39.0 ± 1.6	0.61 ± 0.08
15	2	4.8	18.5 2.8	36.0 5.0	0.51 0.10
16	3	6.0	60.1 2.1	70.9 4.6	0.85 0.07
17	4	6.0	32.7 1.8	52.6 2.0	0.62 0.04
18	5	6.0	33.2 ± 2.1	54.4 ± 2.3	0.61 ± 0.04
Average number for controls:				50.6	

*Litter mates 13 days old were painted with 12 full brush strokes of pure benzene on the right interscapular region of the skin, previously cut. The left side remained untreated and served as control.
†For explanation of symbols *see* Methods of Counting, in the preceding paper.







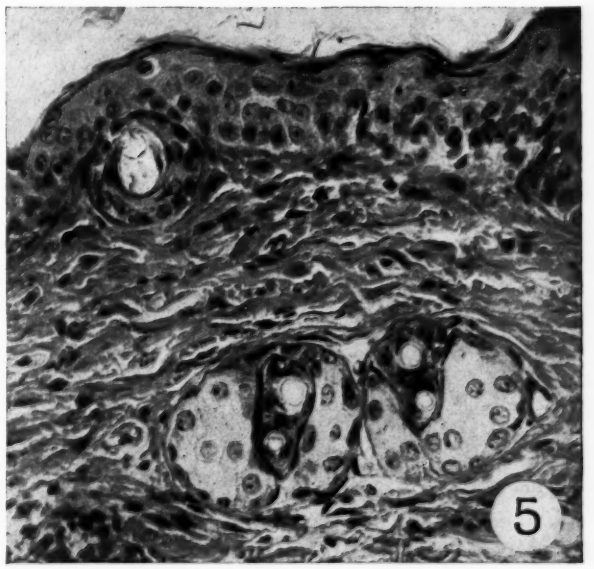


FIG. 2.—Control skin area in mature mouse with normal distribution of mast cells. Toluidine blue. Mag. × 325.

FIG. 3.—The opposite skin area of the same mouse 3 days after one single application of pure benzene. Marked depletion of superficial dermal mast cells. Toluidine blue stain. Mag. × 325.

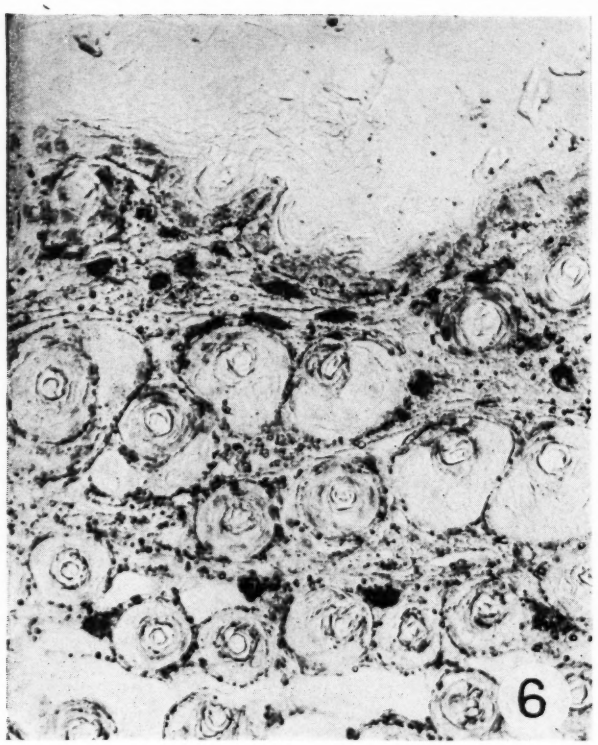
FIG. 4.—Same as Fig. 2. Van Gieson stain. Mag. × 325.

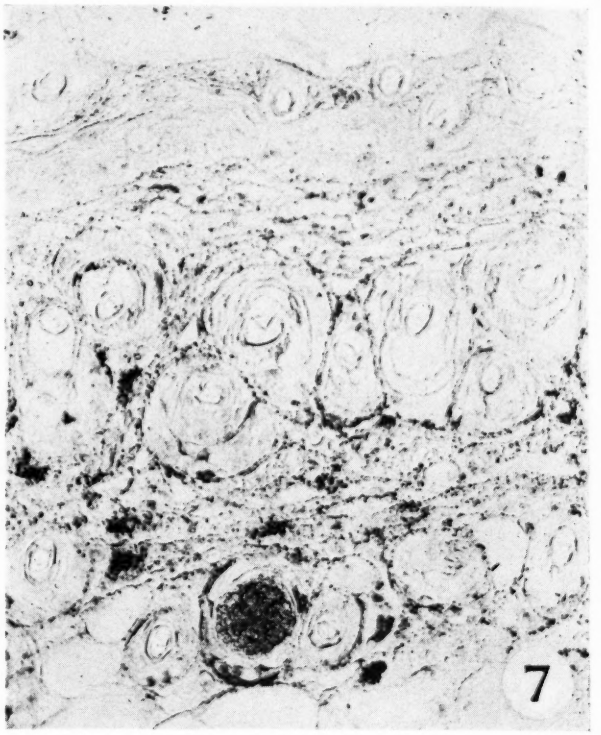
FIG. 5.—Same as Fig. 3. The cytological changes of epidermis and the moderate inflammatory dermal alterations are seen. Van Gieson stain. Mag. × 325.

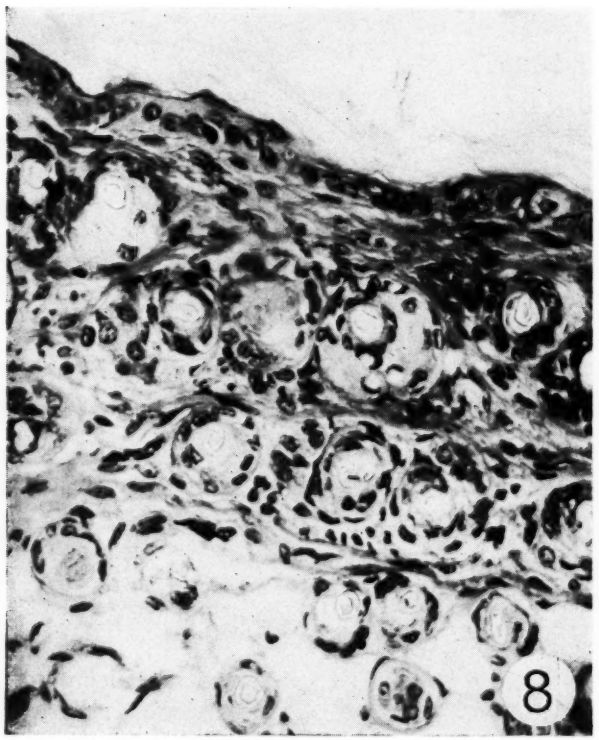
cells. In this series of experiments no signs of metachromatic substances being liberated from the mast cell cytoplasm were seen; no "free chromotrope substances" (14, 16) were found in the dermal connective tissue or in the epidermis coincidental with the loss of granular substance from mast cells; no metachromatic inclusions in connective tissue cell elements or macrophages could be discerned. Nor did the intercellular fluid contain any substances presenting a true metachromatic staining reaction. This was checked by the freezing-drying technic in a small number of cases. In other words, the present investigation could not elucidate the fate of the liberated granular substance.

It may be well to stress that the decrease observed in the mast cell number was strictly limited to the painted skin areas. In the control flaps on the left side of the animals the numbers of mast cells were as great as in unpainted mice (5).

Concerning the quantitative correlation between dosage and the resulting loss of granular substance, these investigations are inconclusive. The maximal loss of visible mast cells for both series is about 50 per cent. Considering our deficient knowledge of the amount of granular







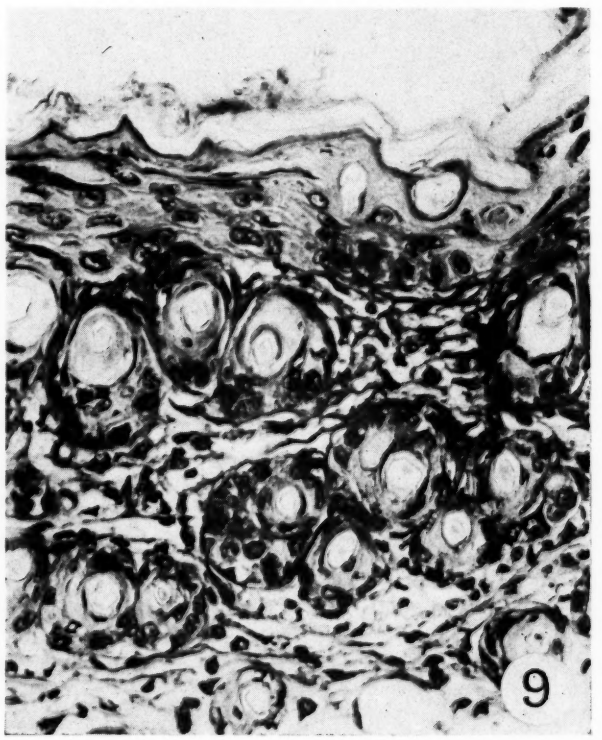


FIG. 6.—Unpainted control skin area from 13 days old mouse. A considerable number of mast cells in the superficial part of dermis. Toluidine blue stain. Mag. × 325.

FIG. 7.—Two days after painting with 12 brush strokes of pure benzene on the right interscapular skin a very marked depletion of mast cell granular substance is seen. Same animal as in Fig. 6. Toluidine blue stain. Mag. × 325.

FIG. 8.—Same as Fig. 6. Van Gieson stain. Mag. × 325.

FIG. 9.—Same as Fig. 7. Pronounced epithelial changes and moderate inflammatory cell reactions on the part of the dermal connective tissue. Van Gieson stain. Mag. × 325.

substance in the mast cells we are not justified in making any definite statements as to the quantitative correlations before comparable data are obtained. These conditions will be further discussed in a subsequent paper.

With a view to the quotient B/b, which refers to the deepest dermal and the hypodermal layers, we have to emphasize its usual instability. Even in normal mice this quotient showed very large individual variations (5), and so we are compelled to state that the values obtained in this series render no simple conclusions possible. The suggestion seems justified that mast cells situated in the hypodermis do not participate in the reaction to benzene painting, at least not under the conditions used in this experiment. Hypodermal mast cells are not included in the count for each millimeter of epidermal length (Tables I and II: C and c).

As far as we know, this early effect of single applications of pure benzene to the skin has been neither observed before nor predicted. With a view to continued research on the biological significance of the granular substance in mast cells and the possible types of reactions in which the constituents of the granules may take part (13-16), it seems to be of importance to consider the nature of the reaction reported above. The suggestion will thus be advanced, that the immediate response to benzene painting is a local depletion of labile sulphur compounds in the skin (1, 2). This depletion is an early reaction, occurring during the first hours after painting, and it may secondarily induce a subsequent series of reactions tending toward the restoration of the sulphur level. The granular substance of the tissue mast cells might achieve this sulphur restoration in the surrounding connective tissue, and these cells might have a role to play in the production of sulphurous compounds to the tissues. These results and their possible significance open new doors to the approach of the mast cell problem, and it seems advisable to undertake more careful studies on the reactions of skin to different chemicals. Further discussions will therefore be postponed until additional data can be presented.

SUMMARY

The morphological response on the part of the tissue mast cells to single applications of some common organic solvents has been investigated in the skin of mixed albino mice. Serial observations of the number of dermal and hypodermal mast cells, together with approximate estimations of their granular content are reported. Chemically inert solvents such as alcohol, ether, and acetone did not induce any significant changes. On the other hand, single paintings with pure benzene resulted in depletion of the metachromatic granular substance of the superficial mast cells. This effect was strictly confined to painted skin areas, and was found to occur late during the first day after painting and to reach a maximum level during the second to fifth day after painting. The suggestion has been advanced that the granular substance is delivered to the skin in order to restore its content of labile sulphurous compounds, which have been depleted during the first hours after painting (1, 2).

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Mast Cells in Experimental Rat Sarcomas*

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The mast cells described by Ehrlich (6) are characterized by granules showing a so-called true metachromasia. Using as a basis Lison's statement (22) that this color reaction is typical of macromolecular sulphuric ester compounds and Jorpes' (17) demonstration that heparin is a mucoitin ester sulphuric acid showing a distinct metachromasia, Holmgren and Wilander (11) proved the mast cell granules to consist of a material with the same properties as heparin. These authors were able to isolate 22 mgm. of active heparin from 10 gm. of mast-cell-rich capsule of cow liver, whereas preparations of mast-cell-poor capsule of lamb liver proved to be inactive. Jorpes, Holmgren and Wilander (19) also confirmed the conception of mast cells as bearers of mucoitin ester sulphuric acid (heparin). Later this was corroborated by Hirth (10). Wilander (33) in a more comprehensive treatise on the nature of heparin demonstrated the mast cells to be the bearers of heparin. Mast cells are found exclusively in connective tissue, in rather variable amounts within the different parts of the body. Ehrlich had already emphasized that these cells occur chiefly in the vicinity of blood vessels. For bibliographical notes pertaining to mast cells, *see* Lehner (20), Holmgren and Wilander (11) and Michels (25).

The pathology of mast cells seems still to be only partially recognized. Maximow (24) states that mast cells change their appearance during inflammation. Some are said to burst, and their granules are found in the tissues. Maximow also emphasizes that the new tissue is free from mast cells. Nakashima agrees with Maximow and stresses the fact that during the degeneration of mast cells the granules lose their metachromasia. In addition, Ernst (8) describes morphologic changes of mast cells during the first hours of the inflammatory process.

The appearance of mast cells in tumors of various kinds has been dealt with by many authors. Sylvén (31) described the appearance of mast cells in sarcoma of connective tissue origin. He emphasized that the largest number of these cells has "always been demonstrated within the peripheral parts of the tumors, where the infiltrative destructive growth and disintegration of surrounding normal tissues takes place." On the other hand, mast cells occur only occasionally in the central parts of the tumor. Sylvén (31) has reviewed the literature on mast cells in mesenchymal tumors.

The fact that several authors (1, 3-5, 9, 21, 26, 28, 29, 32) have pointed out the large numbers of mast cells to be found in the skin of mice with tar cancer is of considerable interest for our work. Mast cells will sometimes even form proper nevi (3, 4, 9, 29). Borrel, Boez and de Coulon, (3) who were of the opinion that cancer is caused by a virus, thought that the mast cell reaction "opened the door to all kinds of infections and thus favoured the development of cancer," whereas Cramer and Simpson (4) believed the accumulation of mast cells to be a defensive process directed against the development of cutaneous cancer.

Cramer and Simpson (4) carried out a thorough investigation of the appearance of mast cells during the development of skin cancer after application of a 0.6 per cent solution of methylcholanthrene in benzene to the dorsal skin of mice. They demonstrated the accumulation of mast cells as a rule to be proportional to the epidermal hyperplasia and considerably to precede the development of malignant growth. In the tumor itself mast cells are scarce but are found in large masses in the immediate neighborhood of the growth, especially where the adjacent epidermis shows advanced hyperplasia. Bloom (2) described the spontaneous appearance of tumor-like masses of mast cells in the skin of older dogs without skin cancer. Cramer and Simpson (4) pointed out that this observation is of interest, as skin cancer is relatively common in dogs.

With regard to human pathology, the appearance of large masses of mast cells in lesions of a fairly rare skin disease, *e.g.* urticaria pigmentosa, should be mentioned. No relationship of this disease to skin cancer is known.

Finally the fact may be recalled that human myeloid leukemia is characterized by an increased number of blood-mast cells, as described by Holmgren and Wohlfart (14). The relative percentage of blood-mast cells may sometimes rise to about

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40 per cent and higher in so-called mast cell leukemia.

In investigations on experimental sarcoma in rats it was noted that the tumors often contained large amounts of mast cells. The results of a study of this subject are presented in this paper.

MATERIALS AND METHODS

In the investigations to be described white rats from a strain bred at the Karolinska Institute were used. At the beginning of the experiments the animals were usually from 2 to 3 months old. In order to produce sarcoma experimentally, one of the following methods was adopted:

I. Subcutaneous implantation of small pieces of 4 per cent methylcholanthrene-cholesterol into the back.

II. Single subcutaneous injection of about 0.1 cc. of a 0.5 per cent olive oil solution of methylcholanthrene into the back.

III. Single subcutaneous injection of about 0.1 cc. of a 0.5 per cent 3,4-benzpyrene olive oil solution into the back.

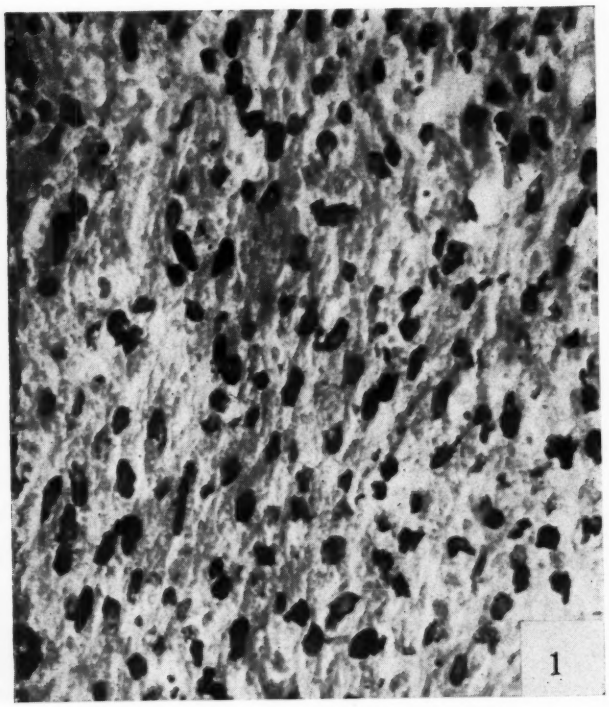
Irrespective of the method used, after 5 to 10 months a localized tumor was often found, which proved histologically to be a sarcoma, although of varying type. In the transplantation experiments small pieces of tumor were implanted subcutaneously into the back by means of a trocar. These transplants often "took" quite easily. In some cases, however the result was somewhat less satisfactory depending on the kind of the transplanted tumor pieces. All the specimens for histological examination were fixed with 10 per cent formaldehyde solution. In some cases 4 per cent basic lead acetate was used, an appropriate fixation for mast cell granules in view of their possible water-solubility (11, 12). When dealing with mast cells of rats, formaldehyde is used with advantage, as the granules hardly dissolve in water. The diffuse tissue metachromasia seems to be more susceptible to this influence, which is counterbalanced by the use of lead acetate. The specimens were embedded as usual and cut in sections 5 to 10 μ thick. The sections were stained with 0.5 per cent toluidine blue aqueous solution. Details of fixation and staining have been described by Holmgren (12) and Sylvén (31)

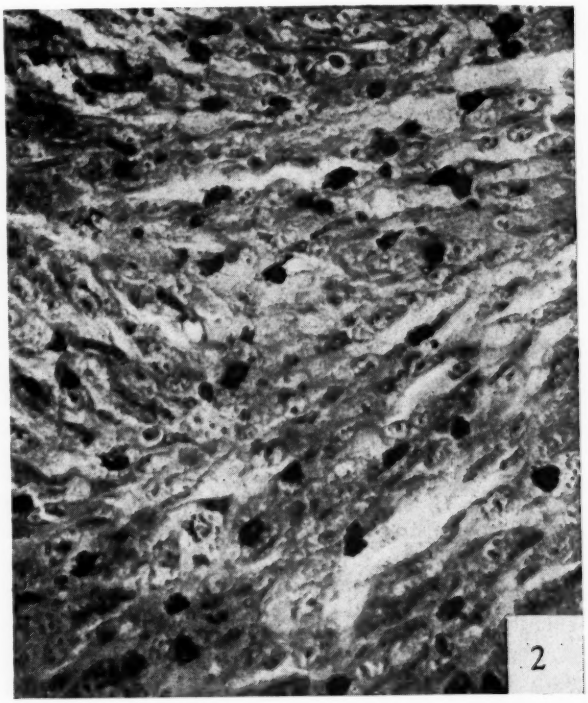
RESULTS

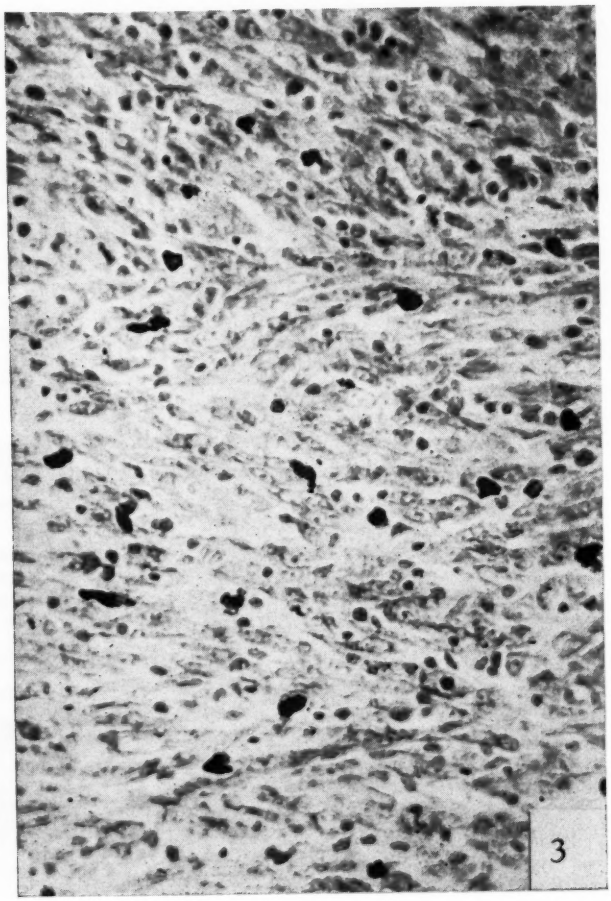
A total of 47 cases of sarcomas experimentally produced in white rats was studied. In 27 cases these sarcomas were produced by injections of methylcholanthrene, in 16 cases by implantation of pieces of methylcholanthrene-cholesterol, and in 4 cases by injection of benzpyrene. All 3 methods resulted in sarcomas of varying types. The majority of the tumors were of polymorphocellular or fibroblastic character. Frequently the type of tumor changed in different portions. In a small number of cases lipomyxomatous and rhabdomyosarcomatous types were observed. When stained with toluidine blue, cells containing metachromatic granules, *i.e.* mast cells, appeared in varying number in all tumors, in the tumor tissue as well as in the capsule. While mast cells occurred rather infrequently or were almost lacking in the polymorphocellular tumor portions (sometimes there were none in several visual fields), they were usually present in large numbers, and sometimes abundantly in the fibroplastic portions (Fig. 1). In the rather infrequently observed lipomyxomatous portions only occasional mast cells were found, whereas their number was somewhat larger in the rhabdomyosarcomatous portions.

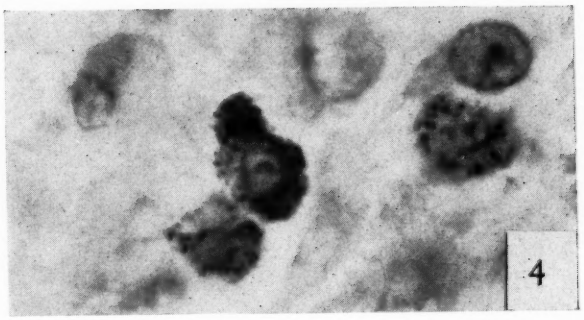
In Figs. 1, 2 and 3 the varying mast cell incidence in fibroblastic sarcoma portions will be seen. Definite interrelation with the intratumoral blood vessels was not noted. Neither did there emerge a connection between the degree of vascularization and the percentage of mast cells. The polymorphocellular sarcomas were, by the way, extraordinarily vascularized.

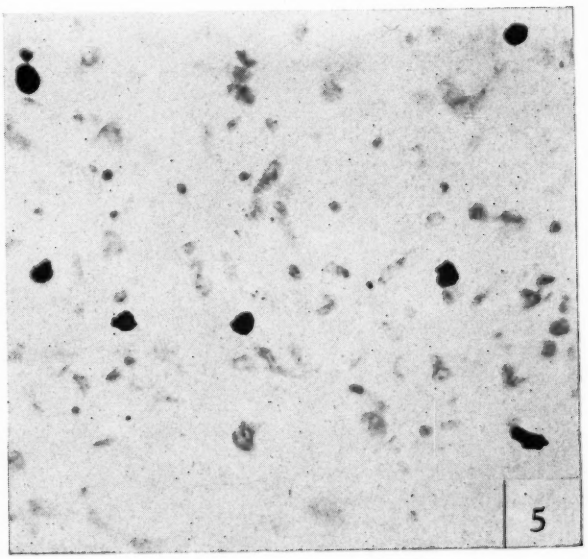
As a rule there was no morphologic difference between the mast cells within the tumors and those situated in other tissues. The content of granules in these cells varied greatly (Fig. 4). Within the frequently occurring necrotic areas, uninjured mast cells were sometimes seen (Fig. 5). This might be taken to indicate a greater resistance of mast cells than of other cells. If mast cells are amoeboid cells, as Lehner (20) and others assume, one might suppose that they invade secondarily the necrotic parts. However, the fact that mast cells as a rule are regularly scattered over the necrotic areas and generally quite as thickly as in the neighboring portions, fails to support this presumption. Sometimes the mast cells situated within the necrotic areas displayed a varying affinity to the stain, so that the granules stained a dark blue or blue-green with toluidine blue. In the most advanced necroses mast cells have completely disappeared or are noticed only as indistinct, considerably degenerated cells. The mast cells with blue to blue-green granules are also found in the normal tumor tissue, although more sparcely. One might blame deficient staining, yet this cannot obtain, since around the cells in question other cells are found with obviously metachromatic granules. According to unpublished observations of Holmgren, these cells occur also under other similar conditions. They are to be regarded as having undergone certain changes.











FIGS. 1-5

Diffuse tissue metachromasia was often noted within the tumors. The same type of diffuse metachromasia has previously been described by Holmgren (13) and others in growing embryonal tissue and was recently found by Holmgren and Rexed (15) in the Büngner bands of regenerating peripheral nerves in rats. Sylvén (31) observed in granulation tissue and in various mesenchymal tumors diffuse metachromasia which he called "free chromotrope substance" and which in his opinion is due to mast cells. This conception is based upon a subjective estimation of the quantity of granules and the number of mast cells in tissue containing much or little free chromotrope substance respectively. Sylvén states that fewer mast cells, poor in granules, are present in the former than in the latter. It is of interest to note that Quensel (27) found in cancer a "mucoid" which stains similarly to mast cells, wherefore he presumes it in both cases to be the same substance.

In this investigation no very close attention was paid to the diffuse tissue metachromasia. As for Sylvén's theory, the fact should be stressed that no obvious connection, whatever, between diffuse tissue metachromasia and mast cells content emerged in our material. One could, for example find quite often that areas with strong diffuse metachromasia also contained a large amount of mast cells. These cells, with variable content of granules appear also in parts without diffuse metachromasia.

The enormous number of mast cells that we found in some tumors raises the question of the histogenesis of mast cells. One might ask oneself whether these cells invaded from adjacent structures or originated from the stroma of the tumor tissue. The experimental sarcomas may grow to an exceedingly large size (in our series we met with tumors weighing over 200 gm.). If these tumors are extremely rich in mast cells, as is quite often the case, the neighboring tissue could be expected to be strikingly poor in mast cells, provided the mast cells invaded the tumor from the vicinity. Examination of the tissues surrounding the tumors, however, always showed a fairly normal mast cell content. In no case did a rough subjective estimation of the mast cell content reveal a difference as compared with normal animals. As already mentioned, the tumor capsules contained a remarkable amount of mast cells both in fibroplastic and polymorphocellular sarcoma. *A priori* the most likely assumption appears to be that the mast cells develop locally in the connective tissue of tumors. The fact that part of the mast cells contain only a few metachromatic granules might also be taken to suggest such an origin. Under these circumstances one would expect to find evidence of mitosis. However, in spite of systematic investigation in no case were signs found indicating mitosis or amitosis. This agrees with the fact that neither Holmgren (15) or others have found mast cells in a state of mitotic or amitotic division in fetuses of rats, mice or man. The appearance of mast cells with only a few metachromatic granules might naturally indicate that these cells had lost most of their granules, and it would be difficult to exclude this possibility in every case. The conception that the granule-poor cells in tumors rich in mast cells might represent young cells where the granules are developing, is supported by a certain similarity of these cells with mast cells in fetuses. There one also finds that the granules appear first in the peripheral parts of the cells as small, sometimes dust-like particles.

In one experiment macroscopically healthy normal tumor pieces were under aseptic conditions transplanted subcutaneously into 10 to 15 young rats. Nearly one-half of the transplanted pieces started growing. From one of the daughter growths new transplantations were carried out in the same way. Thus we followed a tumor through 4 generations, not including the mother growth. Both the mother growth and the various "generations" appeared histologically to be fibrosarcomas. The mother growth contained relatively few mast cells. The first tumor generations presented the same picture as the mother growth and also contained few mast cells. The tumors of the last two generations showed more polymorphous cells and

DESCRIPTION OF FIGURES 1 TO 5

FIG. 1.—Fibrosarcoma from white rat, extremely rich in mast cells, and induced by subcutaneous injection of 3, 4-benzpyrene in olive oil. Toluidine blue stain. Mag. × 250 (approx.).

FIG. 2.—Sarcoma rich in mast cells from white rat induced by subcutaneous injection of methylcholanthrene in olive oil. Toluidine blue stain. Mag. × 400 (approx.).

FIG. 3.—Sarcoma from white rat with fair number of mast cells, induced by subcutaneous injection of methylcholanthrene in olive oil. Toluidine blue stain. Mag. × 250 (approx.).

FIG. 4.—Higher magnification of mast cells in sarcoma from white rat, induced by methylcholanthrene in olive oil. Granulation varies greatly. Toluidine blue stain. Mag. × 1,200 (approx.).

FIG. 5.—Mast cells in necrotic area of sarcoma, produced in white rat by subcutaneous injection of methylcholanthrene in olive oil. Toluidine blue stain. Mag. × 250 (approx.).

more necroses than the first generations. Quite naturally it was difficult to determine the exact number of mast cells owing to the varying distribution in the different areas. Therefore, we always examined several portions of the tumors. On the whole we received the impression that with every new generation the picture became more polymorphous, the number of mast cells simultaneously declining. Other transplantation experiments were carried out only through 2 daughter generations. Nothing of interest emerged in addition to these findings.

From our experiments it follows that mast cells consistently occur in experimental sarcomas of white rats, caused by carcinogenic substances, and especially in those of the fibroblastic type, where they sometimes even reach an excessive development. It is of interest that the mast cells accumulate to a certain extent in the tumor capsules encasing the sarcomas, and in the connective tissue beneath the skin hyperplasias and carcinomas artificially produced by Cramer and Simpson (4). The study of tumors by Sylvén (31) showed that the number of mast cells in his 21 cases of sarcomas of connective tissue origin varied but that the largest amount was found in the periphery of the tumors where "the infiltrative-destructive growth and disintegration of surrounding normal tissues takes place." According to Sylvén, as a rule mast cells are occasionally found in the central parts of the tumors, especially along the vessels. The content of granules in mast cells within the infiltration zone varies widely, and it is not unusual to find only a few granules.

Of course, it is not possible to explain the cause of the high incidence of mast cells in certain experimental sarcomas. One feels rather inclined to the belief that these cells take part in the reaction of the system against tumor cells. If this is the case, however, various types of sarcoma seem to produce a changing reaction in the system. The question whether the varying mast cell content depends upon the degree of differentiation in the various tumor types or upon other factors, must be left in abeyance. The assertion by Brack (3a) that rapidly-growing tumors in the epidermis and in the alimentary canal should contain a large number of mast cells is of interest. It is possible that the organ or tissue in which the tumor grows has some influence on the amount of mast cells in the tumor. Furthermore, the position is complicated by the fact that in some tumors there are alternately areas rich and poor in mast cells without any other difference in their histological appearance. At any rate, the appearance of masses of mast cells seems to characterize certain experimental tumor types. The established fact that mast cell granules consist of the anticoagulant heparin is probably of significance in explaining their appearance in tumors.

The statement of Cramer and Simpson (4) that fixation in formalin dissolves the mast cell granules in rats, resulting in the appearance of granule-poor mast cells is of interest in this connection. Fixation in alcoholformol has not the same effect. In our cases we used lead acetate in addition to formalin. The former coagulates heparin and preserves the mast cell granules in rabbits where they are extremely soluble in other fixation media. In our specimens fixed with lead acetate, we observed the same pictures as in those treated with formalin. Therefore, it does not seem likely that the mast cell granules, which in rats are very resistant to water, can be dissolved (12). The inference of Cramer and Simpson (4), that in rats mast cell granules occur in a water-soluble and an insoluble form, does not seem to be satisfactorily established. Furthermore, the authors state that a powerful mast cell reaction develops after treatment with methylcholanthrene and that "certain groups of mast cells show a strong golden-brown fluorescence." According to others and from our own experience, normal mast cells possess no auto-fluorescence. Since methylcholanthrene, which was applied to the skin, has an auto-fluorescence, it appears more likely that the fluorescent cells observed by Cramer and Simpson (4) were macrophages loaded with substance. Admittedly, the fluorescence of methylcholanthrene is bluish, but we do not know whether this color is changed by the fixation. This possibility was disregarded by Cramer and Simpson (4). In addition, F. Sjöstrand (30) found the macrophages to emit a pronounced fluorescence, a fact that should be borne in mind in this connection. This fact should be considered before accepting the statement of Cramer and Simpson as to the auto-fluorescence of mast cells under the conditions described by them.

SUMMARY

Experimental sarcomas in white rats produced by carcinogenic substances regularly contain mast cells. In such sarcomas of fibroplastic type the development of mast cells can attain an extreme degree.

The mast cells present in experimental sarcomas seem to develop locally in the connective tissue of the tumors, and this suggests that they take part in the reaction of the system against the tumor cells.

Within the necrotic tumor areas the mast cells

are frequently well preserved, a fact that seems to point to these cells possessing a greater power of resistance than the tumor cells.

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Pigmented Precancerous and Cancerous Changes in the Skin

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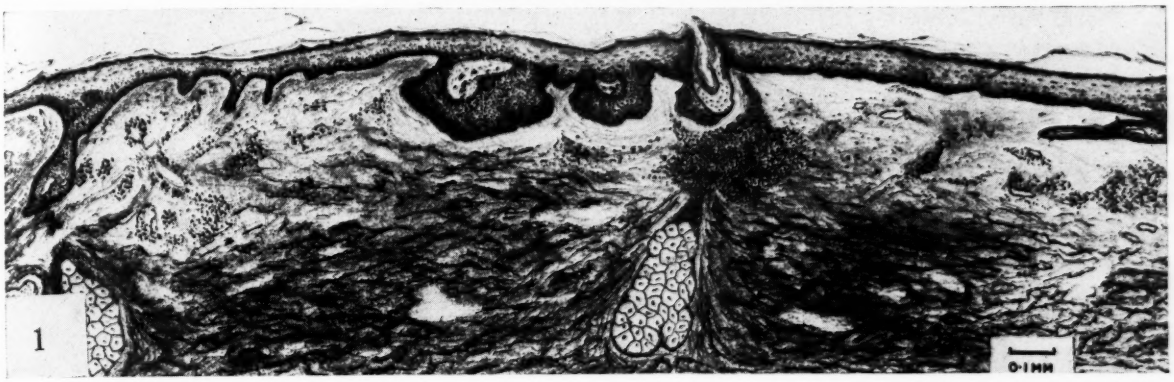
The changes to be described here concern pigmented Bowen's disease, squamous-cell carcinoma and basal-cell carcinoma of skin. They do not include the group of melanoma, melano-epithelioma or melano-carcinoma, nor the pigmentation in conditions sometimes leading to cancer, such as, senile keratosis, keratosis resulting from arsenic, tar or radiation and xeroderma pigmentosum. The changes described in this paper have not attracted enough attention of dermatologists, and Eller and Anderson (8) have stated that pigmented basal cell carcinomas were "quite uncommon." At a recent symposium on "Malignant Melanomata" in Leeds, England (4) the opinion was expressed that, "It was surprising but true, that these (pigmented tumors of epidermal origin) are not recognised by the majority of pathologists, and there is very little in the literature about them. But it is very important that they should be recognised, for the prognosis and treatment in these cases is exactly similar to that of the non-pigmented tumors of the same series and quite different from that of the melanomata. The important point is for the pathologists to recognise that pigment formation is a function of a group of tumors other than the true melanomata". It has been suggested that many melanotic tumors reported to have been cured by local excision or radiation were probably tumors belonging to this group. It is known that some of these tumors originate from pigmented nevi and without a histological examination are not easily distinguished from true melanomas.

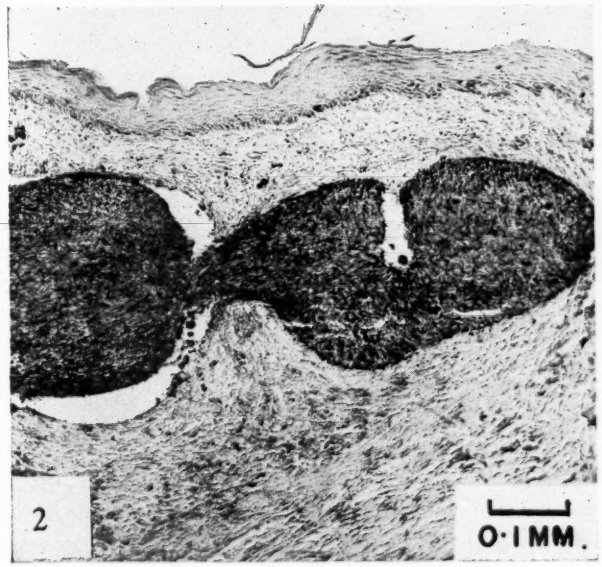
The present study is based on tumors from 15 patients seen by us during the last 5 years at the Tata Memorial Hospital. Four of them showed multiple lesions, and will be considered separately from the rest. The following table summarises information regarding age, sex, location etc. in the remaining 7 out of the first group of 11 cases. These tumors were all deeply pigmented and histologically presented the structure of a basal-cell or a basal-squamous type of carcinoma. They were similar in so many features, that only 4 out of 11 have been described as illustrating their probable mode of evolution.

Case No.	Nationality	Age	Sex	Site	Duration	Diagnosis
1	Muslim	45	M	Chin	1 year	Basal sq. cell ca
2	Parsee	60	M	Scalp	5 years	Basal cell ca
3	Hindu (Deccani)	38	M	Leg	Mole since childhood, recent growth 3 weeks	Basal cell ca
4	Hindu (Gujarati)	54	M	Forehead	8 years	Basal cell ca
5	Parsee	73	M	Nasolabial fold	4 years	Basal sq. cell ca
6	Parsee	59	F	Arm	6 months pigmented mole started bleeding	Basal cell ca
7	European	50	F	Forehead	2 years	Basal sq. cell ca

REPORT OF CASES

Case 8. # 13052.—A 64 year old lean Parsee saw a skin specialist for a fungus infection of his feet and one finger. He also casually referred to an "eruption" on the chest which was diagnosed as a "precancerous condition." The patient was therefore referred to the Tata Memorial Hospital. On examination a roughly oval skin lesion was seen over the right lower ribs in the nipple line. It had started as a small dark spot several years back and had gradually increased in a circular spreading manner. There was neither pain nor itching but a slight oozing of clear fluid from the surface. The lesion was about 5 cm. in diameter. Its edge was raised, about 1 mm. wide, wavy and dark purple in color. It was clearly demarcated from the adjacent normal skin. The central portion was smooth, glazed and pale pink. A cluster of few raised pigmented spots was seen in the center. A biopsy from the edge showed the characters de-





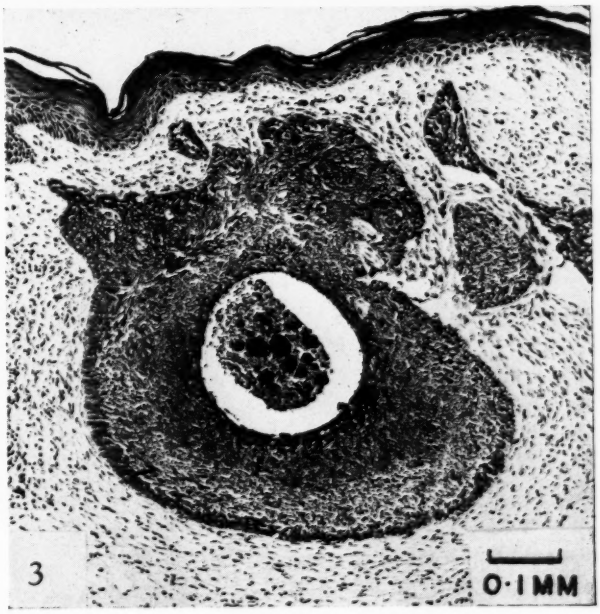


FIG. 1.—Case 8.: A camera lucida drawing of a section of the entire tissue removed by biopsy. The oval bud in the center is over the spreading edge of the lesion; and the healed atrophic skin is seen towards its right. Masson's trichrome stain. Mag. × 65.

FIG. 2.—Case 8.: Photomicrograph showing a network of fine argentophil fibers formed by the numerous dendritic processes of proliferated melanoblasts in epithelial buds. Silver impregnation. Mag. × 100.

FIG. 3.—Case 9.: Photomicrograph showing cords of proliferating epithelial cells pushing into the dermis. The central bud shows an area of degeneration and necrosis in the middle with a debris of pigment granules and desquamated cells. Hematoxylin and eosin stain. Mag. × 100.

scribed below. The lesion regressed rapidly and completely with four exposures of 600 r units daily (85 kv; 1 A1; T. S. D. 15 cms. and a total radiation of 2400 r).

Histological examination.—The biopsy piece (Fig. 1) consisted of stratified squamous epithelium clothing a fibrovascular layer of dermis. The epidermis was thinner than the normal for that region and showed towards the middle of the section a small oval bud of proliferated basal cells pushing downwards in the dermis. The peripheral cells were columnar, contained large oval nuclei and a small amount of pale basophilic cytoplasm. These cells were arranged in a palisade. The more loosely arranged central cells were smaller with round or oval nuclei containing sparse granules of chromatin material. There was a minute area of surface ulceration over the epithelial bud. The epidermis of the normal skin showed widely separated short rete cones. The basal layer of cells was darkly pigmented with fine brown particles. In the healed area, towards the center of the lesion, there was a complete obliteration of rete cones and the epithelium was flat and thin. The basal cells were without pigment. In this area the pars papillaris of the dermis showed a loose, irregular texture of collagen fibrils, interspersed with arcades of newly formed blood capillaries and foci of mononuclear cellular exudate. The pilosebaceous structures were distorted and atrophic. The downgrowing bud was lying in a bed of concentrically arranged lax, edematous collagen

fibrils. Focal accumulations of lymphocytes and histiocytes were lying outside this zone. In sections impregnated with silver the epithelial bud showed the proliferating basal cells interwoven with a large number of melanoblasts connected with a meshwork of fine argentophile fibers (Fig. 2) formed by the numerous dendritic processes of these cells. The elastic tissue net was absent in the superficial zone of the dermis except along the few hair sheaths and ducts of sweat glands. There were few macrophage cells loaded with coarse brown pigment in the dermis. The section gave an impression of healing at the center and a spreading neoplastic edge at the periphery, consisting of proliferated basal cells and melanoblasts. In the healed area the epidermis and the surface zone of the dermis were morphologically altered, but no trace had been left of the neoplastic epidermal cells.

Case 9. #14275.—A fair complexioned Parsee, 47 years old, had two small pigmented moles on his body "ever since he could remember." One was situated over the middle of the right clavicle and the other in front of the upper third of the right arm. The latter was slightly raised above the surface, about the size of a lentil (5 mm.) and surrounded by an areola of brownish skin roughly 2.5 cm. broad. About eight years back the mole on the arm began to grow in size and the surface "broke into scab covered black fragments". Recently the fragments began to itch and weep. He consulted a surgeon who excised the two moles and sent the one from the arm to us for histological investigation.

Microscopic examination showed a picture similar to that of the advancing edge in Case 1, except that there were several separate buds of proliferating basal cells growing deeper down into the dermis. The bigger buds showed a central area of degeneration and necrosis, with a clear space containing a debris of pigment granules and desquamated flakes (Fig. 3). The basal cells tended to flatten as they approached the core. There was no evidence of healing. The melanoblasts appeared to proliferate and migrate away from the periphery of the buds. They were gradually involved in the degenerative process of the cells towards the center. The proliferating buds of epidermal tissue lay in a broad sheath of loosely arranged collagen fibrils. Subjacent to the superficially ulcerated epithelium, there was a rich cellular exudate of lymphocytes, eosinophiles and histiocytes, between richly sprouting blood capillaries.

Case 10. #E788.—An olive-complexioned 63 year old Anglo-Indian physician had a small hairless mole on his right forearm lateral to the flexor tendons, about 6 cms. above the wrist for "many, many years." The color of the mole was uniformly black and it was smooth on the surface. About two months back he noticed that the mole had begun to increase in size and to itch. The physician attributed this to long hours spent every day in filling a multitude of army forms. He remembered that the itching sensation began one night and the next morning there was a slight erythematous area round the mole. The surface became rough and raised with a couple of weeping points exuding a clear pinkish fluid. No scabs were formed. As the mole rapidly doubled its size, the physician consulted a surgeon who excised the mole and gave him a rather gloomy prognosis about his condition. He saw us with his excised tissue.

A naked-eye examination of the tissue showed a superficially ulcerated, small (6 mm.) brown nodule raised about 3 mm. above the surface. On cut section an ovoid, dark brown, firm mass clearly stood out from the dermis. It was darker near its outer edges. The microscopic examination revealed a sharply circumscribed nodule of neoplastic cells (Fig. 4), composed of lobules separated by filamentous processes of fibrovascular connective tissue. The cords and lobes presented the characters of a baso-squamous epithelioma (Fig. 5) with round or oval spaces in the center filled with fine concentrically arranged lamellae of keratinized material. An area of surface ulceration was dipping into dilated follicles, crypts and fissures burrowing into the tumor mass. The tumor cells in the peripheral cords were unevenly laden with dark brownish pigment. The silver preparation (Fig. 6) showed a proliferation of melano-

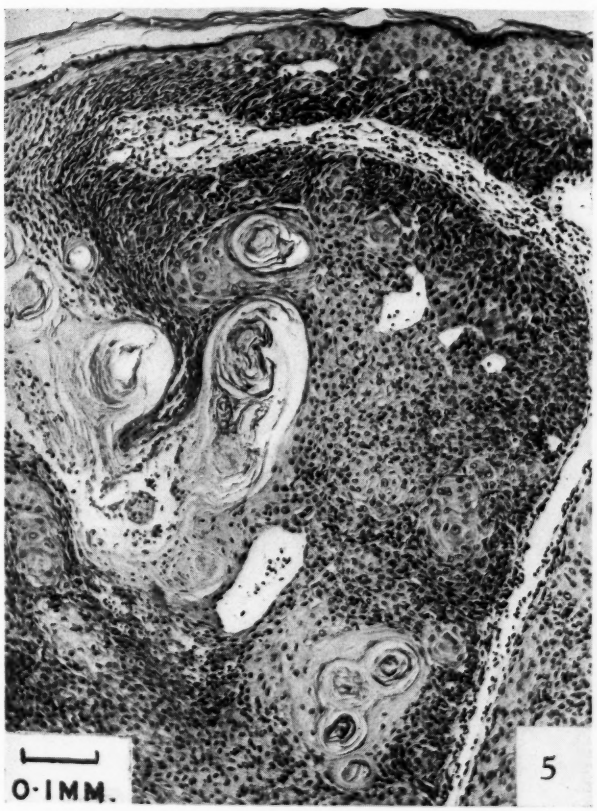
DESCRIPTION OF FIGURES 4 TO 7

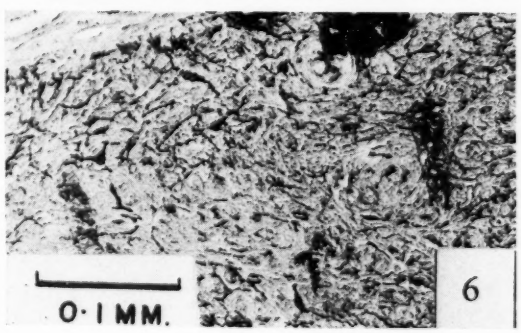
FIG. 4.—Case 10.: A camera lucida drawing of a section of the nodule removed by operation. Faintly stained with hematoxylin. Mag. × 25.

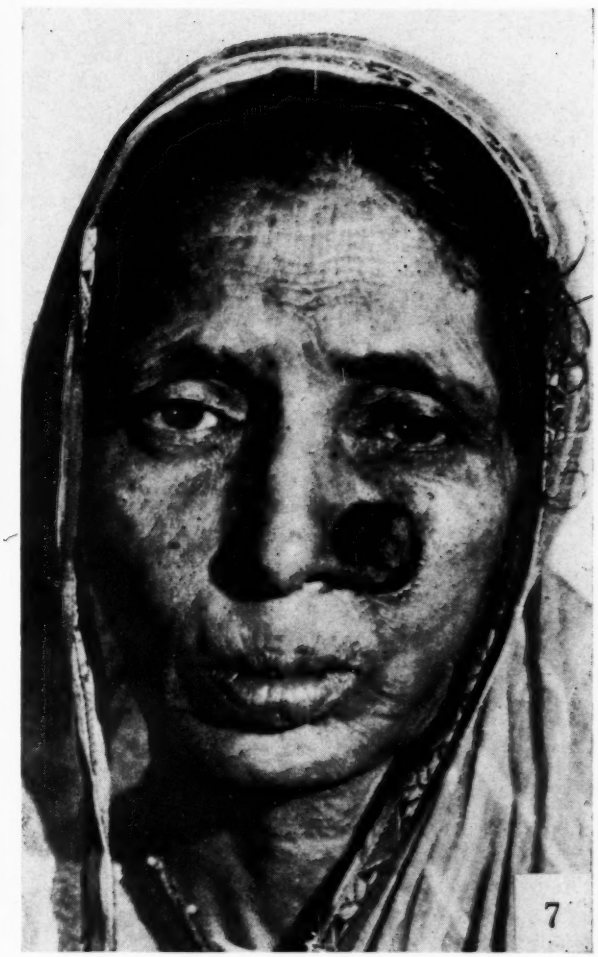
FIG. 5.—Case 10.: Low power photomicrograph of the area marked in Fig. 4 above, showing the characters of a baso-squamous epithelioma. Hematoxylin and eosin stain. Mag. × 150.

FIG. 6.—Case 10.: Low power photomicrograph of a portion of the area marked in Fig. 4 above, showing a proliferation of melanoblasts, with a network composed of their branching protoplasmic processes. Silver impregnation. Mag. × 200.

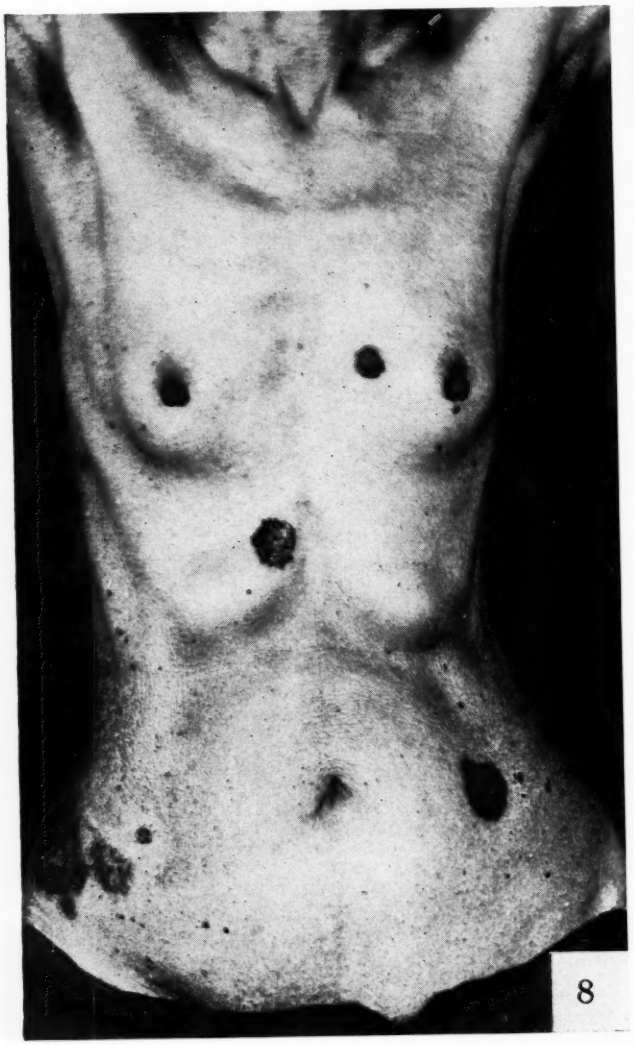
FIG. 7.—Case 11.: Photograph showing a superficially ulcerated dark nodule on the face.







FIGS. 4-7



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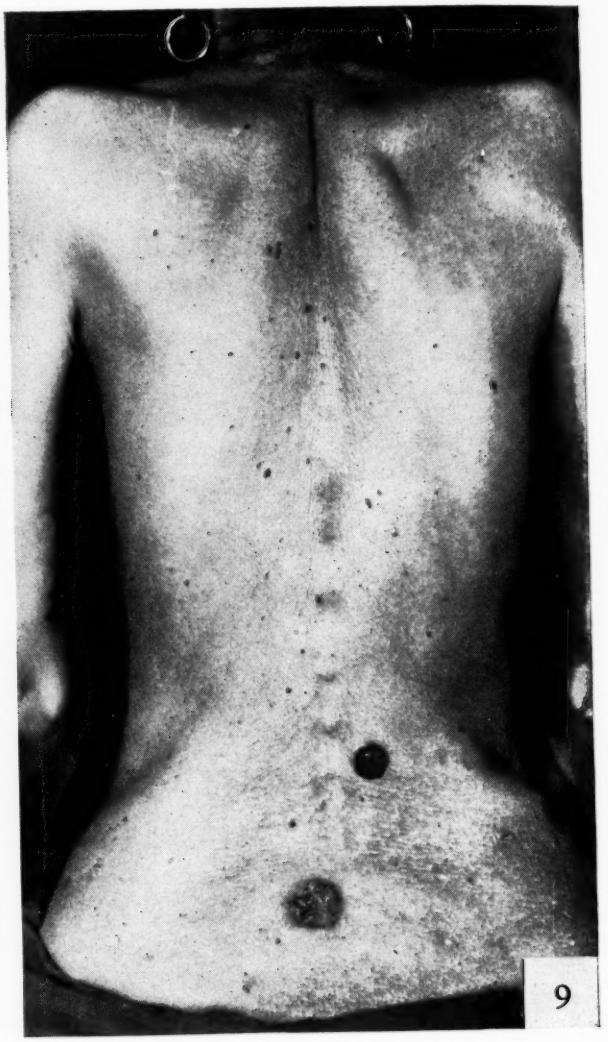

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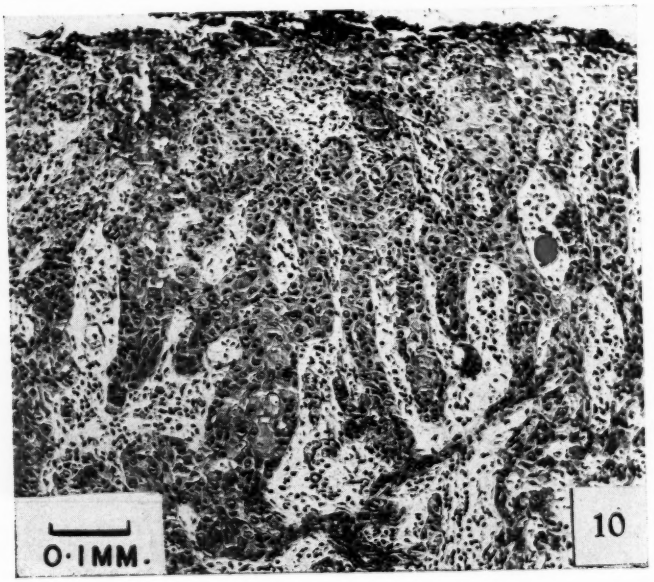

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FIGS. 8-10

blasts and their branching twigs creeping between tumor cells, as well as coarse grains of pigment in groups of melanophores in the dermis.

Case 11. #5220.—A medium colored, 40 year old Mahar woman, mother of two children, was admitted for a small circular superficially ulcerating black growth on the face (Fig. 7). She had noticed the growth for nearly two years. It had started as a tiny sore spot, which did not cause any discomfort except that it occasionally irritated her. She accidently injured it two months back and it began to grow rapidly. On examination, a flat black round nodule about 0.5 cm. in diameter was discovered filling the left nasogonial fold. It was slightly raised above the surface (4 mm.) and was not adherent to the subcutaneous structures. There were a couple of spots of surface ulceration. At its periphery the nodule showed a peculiar smooth, translucent appearance which is often noticed in basal cell tumors of the skin.

Microscopic examination of the excised nodule showed a slightly more advanced stage of basal cell carcinoma, dotted with small cystic spaces containing pigment granules and remains of dead tumor cells. The cells inside the tumor cords were elongated and fusiform. Many of them contained a fine dust of brown pigment in their cytoplasm. Golden brownish pigment was also seen in the bodies and branches of the many ramifying cells between the characteristic columnar and fusiform cells. The dendritic cells were easily discernible in unstained sections. There were also groups of melanophores loaded with clumps of pigment in the dermis.

Comment

All the tumors belonging to this group were characterized by an insidious onset, and a slow clinical course. Many of them were stated to have originated in a pigmented mole which had been present for a very long time. The tumor had sometimes attracted the attention of the patient after a negligible injury. There was no infiltration of deeper structures nor was there an involvement of regional lymph nodes or distant viscera in any of the cases. There was no preponderance of occurrence in either sex. Their location was not restricted to any particular part of the skin, although the face and particularly the nasogonial fold appeared favorite sites. The histological findings in these tumors presented several characteristics in common, and the differences were mainly quantitative as regards (a) the size of the lesion and its encroachment on the dermis (b) the relative participation of polygonal prickle cells in the cords of basal cell carcinoma and (c) the amount of pigment visible or demonstrable in the tumor mass. In none of these tumors true nevus-cell accumulations (cell nests, theques) were seen in the dermis and there was nothing to suggest an affiliation of these tumors with the group of benign or malignant melanomas. These tumors appeared to be satisfactorily eradicated by adequate excision or contact radiation therapy.

Multiple Basal-cell Pigmented Tumors

Case 12. #14612.—A tall, thin, nervous, wheat-coloured, 51-year old Eurasian married woman was admitted for ulcerated black nodules on the skin. When she was about 30 years old a group of "pigmented moles" reappeared on her body. Some of these had begun to spread into black patches during the last 7 to 8 years. She had developed a "boil" above the pubes which burst and formed a red tumor about ¾ inches in diameter. It was excised and treated by a surgeon who examined it microscopically and had called it a "rodent ulcer". She was referred for her black patches to a specialist whom she saw after 5 years. The dermatologist found some areas with scabs on the surface. "One old one looked melanotic. This she had for 15 years. The others have been there for 6 years." As he was of opinion that "the appearance of some lesions was like a precancerous condition" and others like that of a "basal cell carcinoma," the patient was referred to the Tata Memorial Hospital. On examination the woman (Figs. 8 and 9) was found to have numerous (over 200) pigmented moles on the neck, the trunk and the thighs. There were no pigmented spots on the face. There were several flat black patches on the trunk which were roughly circular and clearly demarcated from the neighboring healthy skin. The central portions of these patches were superficially ulcerated and were covered with brownish scales of dried secretion. There was also an intensely black nodule on the back, at the waist line

DESCRIPTION OF FIGURES 8 TO 10

Figs. 8 and 9.—Case 12.: Front and back views of the trunk showing numerous pigmented moles, two superficially ulcerated areas, and three intense black nodules on the skin.

Fig. 10.—Case 12.: Low power photomicrograph from a section from the flat patch behind the right thigh, showing superficial ulceration and thin branching cords of tumor cells infiltrating into the dermis. Hematoxylin and eosin stain. Mag. × 150.

(2 × 1.5 × 1 cm.) and a flat, superficially ulcerated, slightly pigmented patch on the back of the right thigh (1.5 cm. in diameter). These last two were excised, and a biopsy taken from the edge of the patch on the right loin.

Microscopic examination.—The material obtained after excision was available for study and showed a great variety of structure of basal cell carcinoma type. Curiously enough the flat patch behind the right thigh showed greater anaplasia and deeper infiltration by tumor cells (Fig. 10), which were arranged in long thin branching strands, or scattered in small groups of cells in the dermis. The pigmentation was associated with a proliferation of melanoblasts and other changes that have already been described.

Case 13. #3377.—A dark-skinned 76 year old Indian Christian, father of 12 children, was admitted for an ulcerated black nodule over the left lower jaw. He had had a "boil" in the same place when he was 20 years old which had healed after being incised to let the pus out. He developed a small lump (Fig. 11) on the same spot forty years later which had burst and left an ulcer. This ulcer had steadily increased in the course of the previous 15 years, during which time he had developed similar lesions over other parts of the body. The latest of these was about three years old. The ulcer or the lumps did not pain him but he felt an itching sensation over them. On examination a black, firm, flattened mass (3 × 2 × 0.5 cms.) was felt in the skin over the body of the left mandible just in front of its angle. The mass was ovoid, raised above the surface and ulcerated in the center. The surface of the ulcer was covered with dried black scabs. It was freely movable over the subjacent structures. There were three other similar nodules on the trunk. These stood out more prominantly and were less pigmented than the lump on the jaw. They were located over the tip of the left costal cartilage in the right groin and on the left thigh near the attachment of the scrotum. On careful examination, a rhomboid, rough, dry, scaly area on the skin of the left lower abdomen (Fig. 12) was seen that had not attracted the attention of the patient. The area was 4 cm. long at its widest extent and was clearly defined by a black, uneven margin. All these lesions were excised with about 1 cm. of normal skin beyond them and were available for histo-

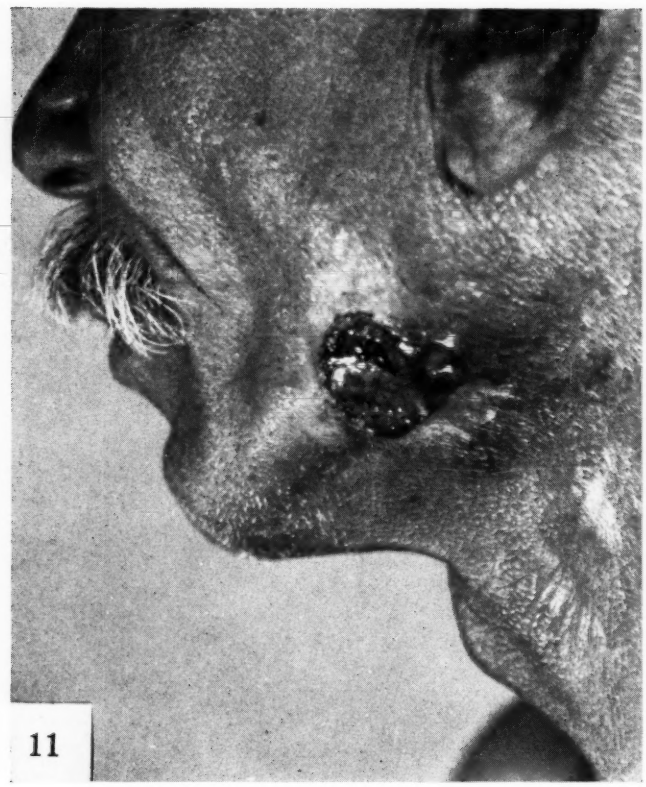


FIG. 11.—Case 13.: Photograph showing the pigmented ulcerating nodule on the left lower jaw.

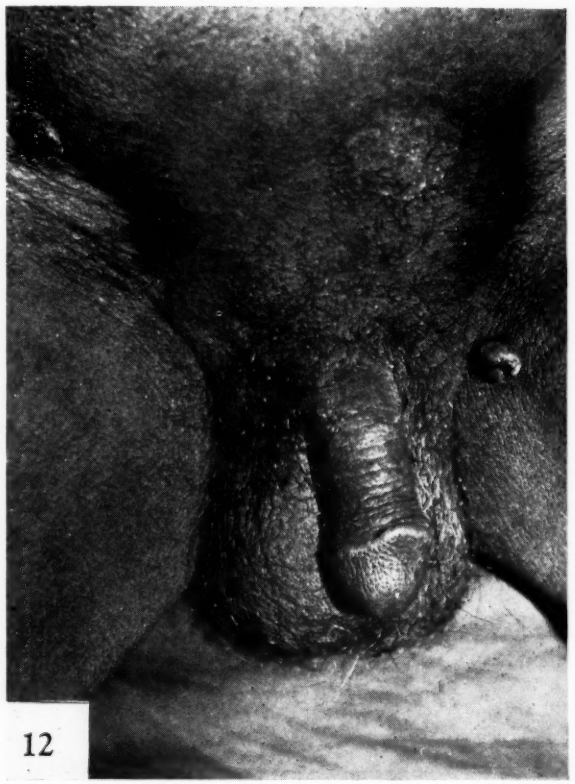


FIG. 12.—Case 13.: Photograph showing pigmented nodules in the two groins and the rhomboid dry scaly area in the left hypogastric region.

logical study. A full-thickness skin graft was placed on the raw area left on the face after the excision of the mass. The patient made an uneventful recovery and has not reported since with any recurrence of his disease.

Miscroscopic examination.—All the lesions including the dry, scaly area showed the structure of a basal cell carcinoma, with slight variations in type in the different nodules. These lesions afforded excellent material for a study of the proliferative changes in melanoblasts, by dopa reaction and silver impregnation. These changes will be referred to later while considering the nature of pigmentation in these tumors.

Comment

Multiple tumors of this type are very rare in published reports. The cases reported by Nomland (16), Pautrier and Archambaut (17), and by Nisbet (15) probably belong in this category. In view of the discussion following the case reported by Nomland (19), it is necessary to point out that the areas of predilection for epithelioma adenoides cysticum, *viz.* the lower eyelids, nose and portions of the cheek, were exempt from disease in both the patients described above. Case 13 was a male and there was no evidence of a familial tendency to disease in either case. Further it was found that even though the lesions in both patients were present for a long period the onset of disease was not in early life, nor was an accelerated growth associated with the time of puberty. The similarity between these cases and those reported in the literature consisted in the following features:

1. A clinical resemblance to pigmented nevi without the presence of nevus cells arranged in cell nests (theques), or strands in the dermis.

2. A slow evolution with probable origin in a preexisting pigmented mole. An absence of regional or distant metastases even after many years' existence.

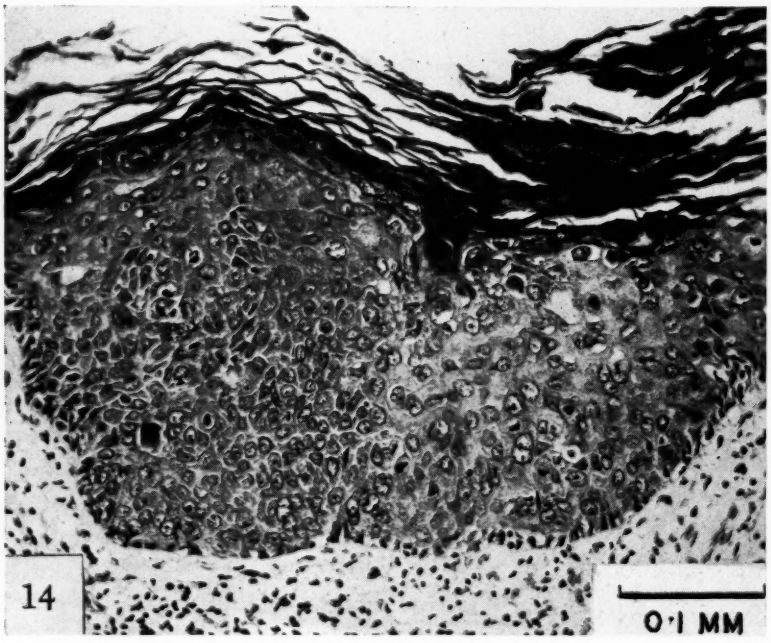
3. Variation in form, color and histological details in different lesions in the same person.

Multiple Squamous Cell Pigmented Tumors

Case 14. #5608.—A fair-skinned Hindu bania, owner of an electrical appliances shop, was admitted to the hospital on Nov. 16, 1943 with an ulcerated growth at the base of the left palm. He gave the following history about his complaint. A few small dark spots had suddenly appeared at the root of the palm just below the middle of the right wrist 10 years previously. These spots increased slowly in size and had fused to form a firm, dark nodule causing itching and some discomfort. He was treated by his physician with ultraviolet rays which seemed to arrest the growth of the nodule. It started growing again after 2 years. He was treated by "application of radium." The condition improved under this treatment. The lesion became active again after an interval of two years. He was treated with deep x-rays in 1937 and 1940. He denied having had any medicines containing arsenic. He was unable to supply exact information regarding the dosage of the x-ray and radium therapy. On examination a firm ulcerating growth about 5 cm. in diameter was seen in front of the wrist spreading on to the palm of the hand (Fig. 13). The growth was surrounded by a broad zone of depigmented skin and a darkly pigmented ring outside it. The growth was partly fixed to the tendons of the flexor muscles. There was a black rough patch with uneven fissured surface on the right wrist. This was excised and showed closely packed black papillary proliferations of the skin moulded on thin strands of connective tissue. A similar lighter patch was present on the palmar surface of the right middle finger. Both palms showed numerous minute discrete translucent nodules in the skin. A small node was felt in the left axilla. The physical examination revealed no other abnormality except a number of large and small *cafe au lait* spots on the back, chest, abdomen and scalp. The face was free from any blemishes. x-ray examination showed marked decalcification of the bones subjacent to the ulcer but there was no destruction or evidence of involvement of bone structures.

The lesion was treated with deep x-radiation. A total dose of 4000 r units was administered to him during the course of 10 days (85 kv. 1 Al, T. S. D. 15 cm. circular field 6 cm. diameter). The ulcer rapidly healed and the wrist movements improved under treatment. At a follow-up 2 months later it was noticed that he had residual disease and he was given a further total dose of 1500 r units over a period of 1 week (200 kv. 0.5 cu + Al, T. S. D. 50 cms. circular field 5 cms. in diameter). The lesion on the palm became cleaner, but some disease still persisted. One month later the patient appeared with a raised pigmented lesion on the scalp, 2 cm. in diameter which bled easily on touching. This lesion and those on the right hand regressed completely with x-ray therapy, but the lesion on the palm persisted. It was therefore decided to amputate the limb at the middle of the forearm.

The patient returned after six months with a small pigmented papillary lesion on the scrotum and again four months later with similar small lesions on the right thumb and the fingers of the



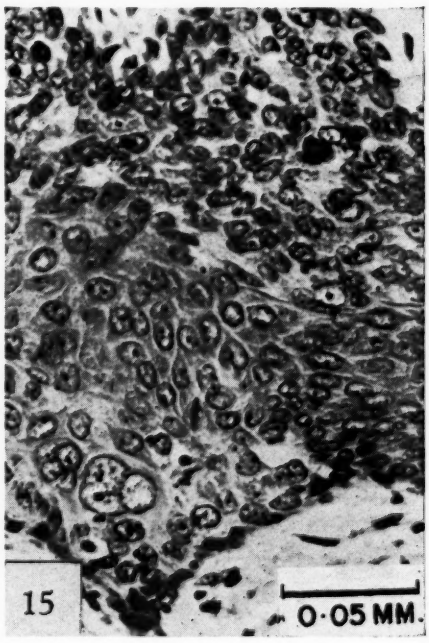


FIG. 13.—Case 14.: Photograph of the palms showing the malignant lesion on the left hand, the darkly pigmented patches on the right, and the minute translucent whitish nodules in the skin covering the palmar surface of the fingers on both hands.

FIG. 14.—Case 14.: High power photomicrograph from one of the minute discrete nodules showing hyperkeratosis, disorganisation of the normal arrangement and stratification of cells, and individual cell keratinisation. Hematoxylin and eosin stain. Mag. × 200.

FIG. 15.—Case 14.: A higher power photomicrograph of a nodule similar to that shown in Fig. 14 presenting the clumping of nuclei, *corps ronds* and monstrous cells. Hematoxylin and eosin stain. Mag. × 375.

right hand. All these lesions rapidly regressed with contact x-ray treatment for 10 days giving a total dose of 4800 r units (50 kv. 1 Al and 4 cm. T. S. D.)

Microscopic examination.—The left hand and the excised lesion from the right hand (b) were available for histological study. The growth on the left palm showed the characteristics of a squamous carcinoma grade III. The pigmented piece excised from the right hand was made up of dermal papillae extended into long branched processes covered with altered stratified squamous epithelium. There was hyperkeratosis, a disorderly arrangement of the cells in the stratified malpighian layer and large monstrous cells with two or three hyperchromatic nuclei. Some cells showed hydropic vacuolation of cells with small pyknotic nuclei. The basal layer of cells was regular, intact, sharply demarcated from the subjacent fibrovascular connective tissue. The small greyish nodules on the palm of the left hand showed the following interesting features.

The surface was covered with several layers of fine lamellae of keratinized material. The epithelium indented the dermis unevenly due to elongation broadening and fusion of rete cones. The minute discrete nodules which were visible to the naked eye were composed of broad epithelial buds pushing into the dermis (Figs. 14 and 15). The cells in the nodule showed a disorganization of the normal arrangement and stratification of cells. There was a wide variation in the size of adjoining cells, with some monstrous cells, and others with several nuclei clumped together, interspersed between normal polygonal cells. Some of the cells showed a characteristic intracellular vacuolation with a preservation of intercellular bridges. In the substance of the neoplastic mass "individual cell keratinisation" (13) and "corps ronds and grains" (7) were seen. These features suggested the change to be Bowenoid in character. It seemed probable that arsenic might have been administered during the course of the varied treatments received by the patient. The skin from the amputated forearm and the hand was therefore analyzed. It gave the following interesting data:

ARSENIC CONTENT OF THE TISSUES

Tissue	Arsenic (as As_2O_3) mgm. per 100 gm.	
Healthy skin in Indians*	0.150	(0.125 minimum) (0.175 maximum)
Normal-looking skin from the forearm of the patient†	0.198	
Cancer tissue from the wrist†	0.924	
Skin containing hyperkeratotic nodules from the palm†	3.036	

*From data published by Bagchi and Ganguly (1).
†Technic employed by Maechling and Flinn (10).

Case 15. #2936.—An elderly emaciated Hindu beggar woman, 60 years old, was admitted for a black warty growth on her abdomen. She stated that the growth had started as an intense black spot 7 years earlier and that she was sure that it was not there before that time. The spot had slowly increased in size and had became rough on the surface. Black warty excrescences had slowly grown out of it. She complained of much itching, and occasional bleeding after scratching. On examination, a verrucous mass, roughly lozenge-shaped (17 × 12 cm.) was seen covering most of the left lower abdominal wall (Fig. 16). The edges were serpigenous and stood out clearly from the adjacent normal skin by their deeply pigmented color and elevated contour. The main mass was deep black except towards the middle where it was depigmented and atrophic in places. A second smaller pigmented patch composed of bunches of large and small papillae was discovered in the right loin. The patient did not remember when it had started. The larger growth emitted a faint fetid odor and the warty projections could be pealed off with little trouble and slight bleeding. Clinical examination and skiagraphic studies did not reveal any morbid condition in the gastro-intestinal tract. Both lesions were excised along with a narrow margin of normal skin beyond the pigmented border. Skin from the thigh was grafted on the raw excised surface of the bigger lesion. The patient made an uneventful recovery. She has not reported a recurrence of the lesion nor of any fresh outcrops of pigmented spots for the last four years.

Microscopic examination.—(a) The flat heavily pigmented peripheral portion of the tumor mass showed a thickening of the epidermis with broadening, elongation and fusion of rete cones. There was hyperkeratosis and parakeratosis of the epithelial layers. In the thickened epidermis there was a loss of normal stratification of cell layers. Many cells in the malpighian layer were swollen, edematous and vacuolated. The nuclei of several cells were large, hyperchromatic and clumped in groups of three or four. There were numerous dyskeratotic cells with pyknotic nuclei and markedly acidophilic cytoplasm. These cells were always surrounded by a clear space. The basal layer of the stratum germinativum was heavily pigmented. There were numerous diffusely pigmented branching cells scattered between the epidermal cells. In the superficial layers of the dermis there were several melanophores stuffed with pigment, besides numerous clumps of free pigment scattered between connective tissue fibers. The transition between the normal and the affected skin was sharp and sudden.

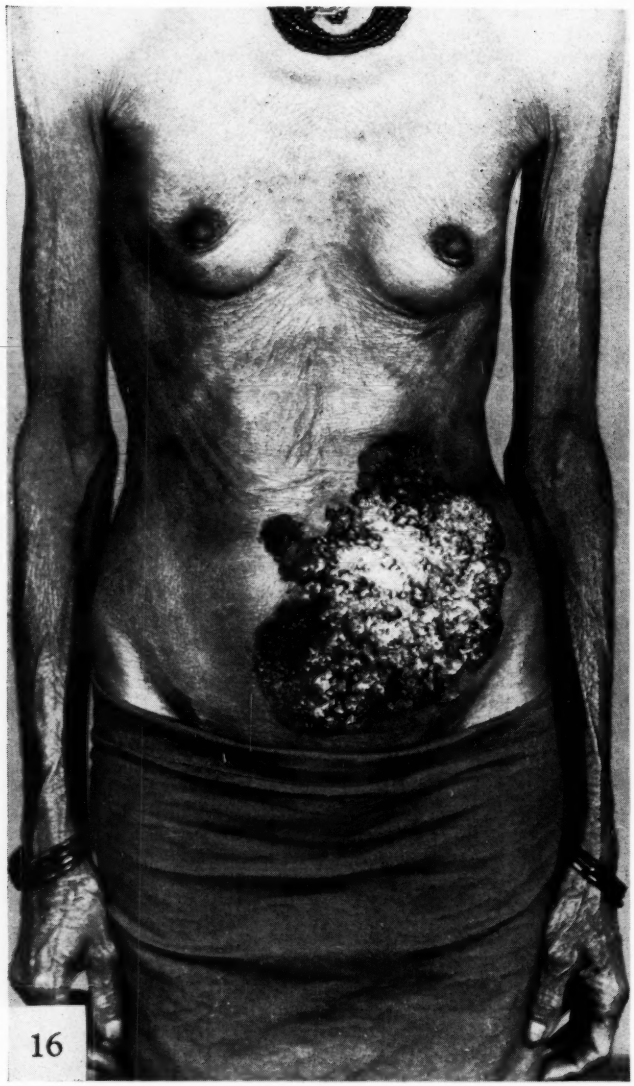


FIG. 16.—Case 15.: Photograph showing the lozenge-shaped verrucous pigmented mass on the left lower abdomen.

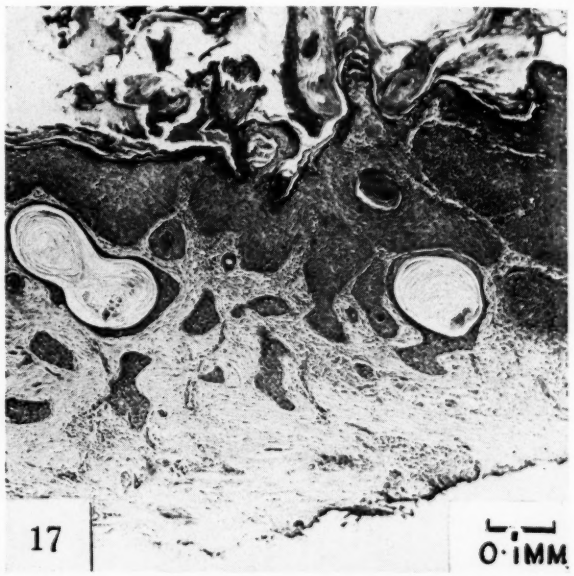


FIG. 17.—Case 15.: Low power photomicrograph showing cords and strands of proliferated epithelial cells with central areas of keratinisation infiltrating the subjacent tissue. Hematoxylin and eosin stain. Mag. × 80.

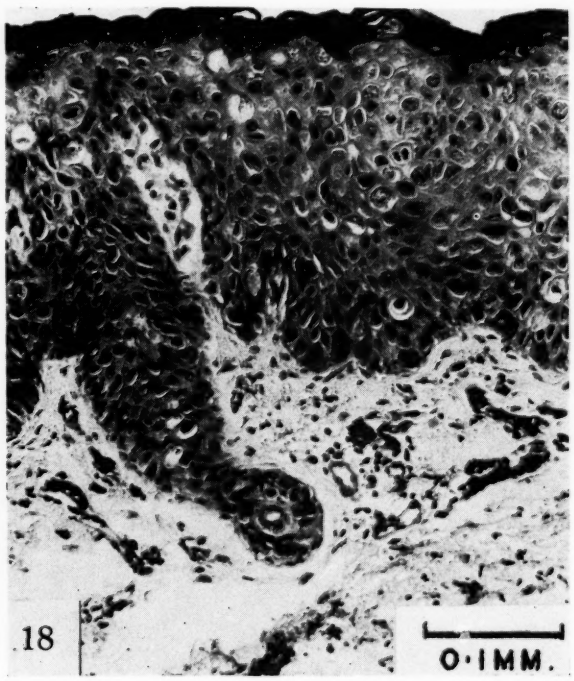


FIG. 18.—Case 15.: A higher power photomicrograph showing the thickened walls of a sweat gland duct and the adjacent epithelium presenting the characters of an intra-epithelial carcinoma. Hematoxylin and eosin stain. Mag. × 200.

(b) The intermediate zone showed several branched filamentous epidermal processes which were covered with several layers of closely applied keratinized lamellae. The epithelium was thickened by an increase in the cell layers and a more pronounced Bowenoid alteration in the character of cells. The peripheral processes were deeply pigmented whereas the more centrally placed filaments of epithelial cells were completely devoid of pigment.

(c) The central portion of the lesion shows a relative thinning of the epidermis accompanied by an invasive proliferation of epithelial cells (Fig. 17) into the subjacent dermis. The proliferating

cells were arranged as cords or strands in the dermis with the development of characteristic epithelial pearls. In some areas the cells of the stratum malpighi showed hyperplastic prickle cells without any of the Bowenoid changes described above. There was a well developed stroma reaction in the dermis mainly consisting of lymphocytes, with few histiocytes and newly formed blood capillaries. The remarkable feature of this area was a complete lack of pigment in epithelial cells and an absence of the branching melanoblasts. There were no melanophores or free pigment clumps in the dermis.

The ducts of the sweat glands (Fig. 18) and the hair follicles in all these sections were involved in the neoplastic process without being altered. The lining of some ducts in the central area however showed the characters of an intra-epithelial carcinoma. The elastic tissue was pushed deeper in all these sections by the inflammatory exudate and the newly formed connective tissue stroma underlying the altered epithelium. The dopa reagent evinced an intense positive reaction in the proliferated and migrating dendritic cells in the peripheral pigmented regions of the tumor mass.

The histological study of the tumor tissue suggests that it belongs to a type of Bowenoid dermatosis, beginning as a deeply pigmented patch which becomes depigmented in the central older area. The depigmented area is characterized by the development of a slowly invading carcinoma of the prickle-cell variety.

Comment

The last two cases show the development of a Bowenoid change in the epidermis antecedent to an invasive cell proliferation and the formation of a typical squamous-cell carcinoma. The interesting feature of these cases is the melanotic pigmentation of the tumor tissue. Bloch (3) had suggested that in the basal cell tumor described by him the pigmentation was the essential feature and that the rest of the structure was secondary. Subsequent pigmentation in patches of Bowen's disease of the skin has often been described, but in both the patients referred to here, the lesions started as black patches and the growing peripheral areas of the fully developed tumor were deeply pigmented as a result of the proliferation and activity of dendritic melanoblasts. This activity and proliferation did not keep pace with the neoplastic growth of epithelial cells. The older, central, fully developed portions of the tumor therefore remained unprovided with pigment-elaborating cells, and became colorless.

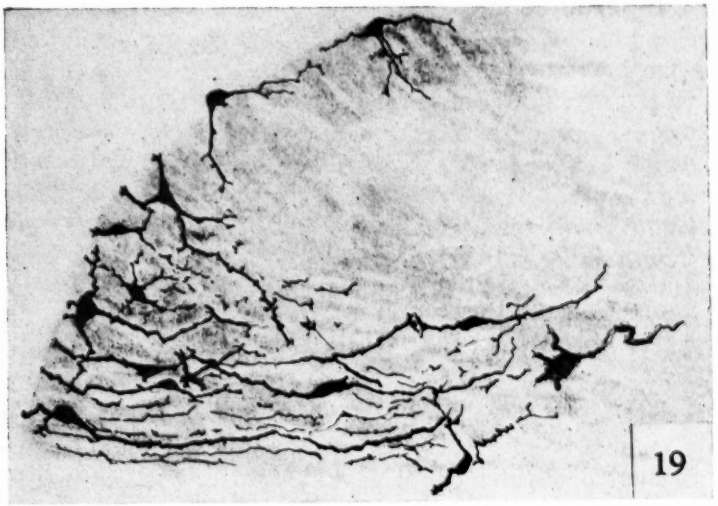
General Considerations

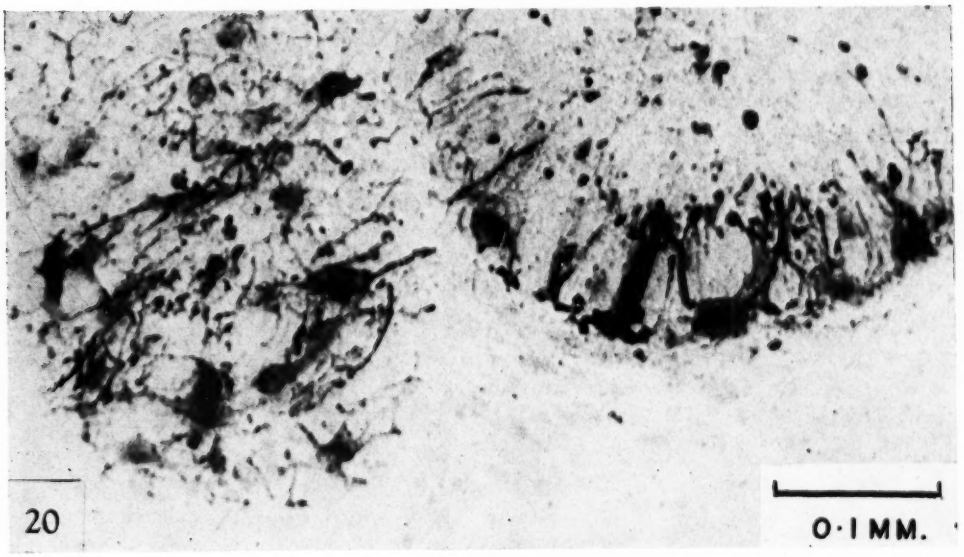
I. Deposition of Pigment in Tumors of the Epidermis

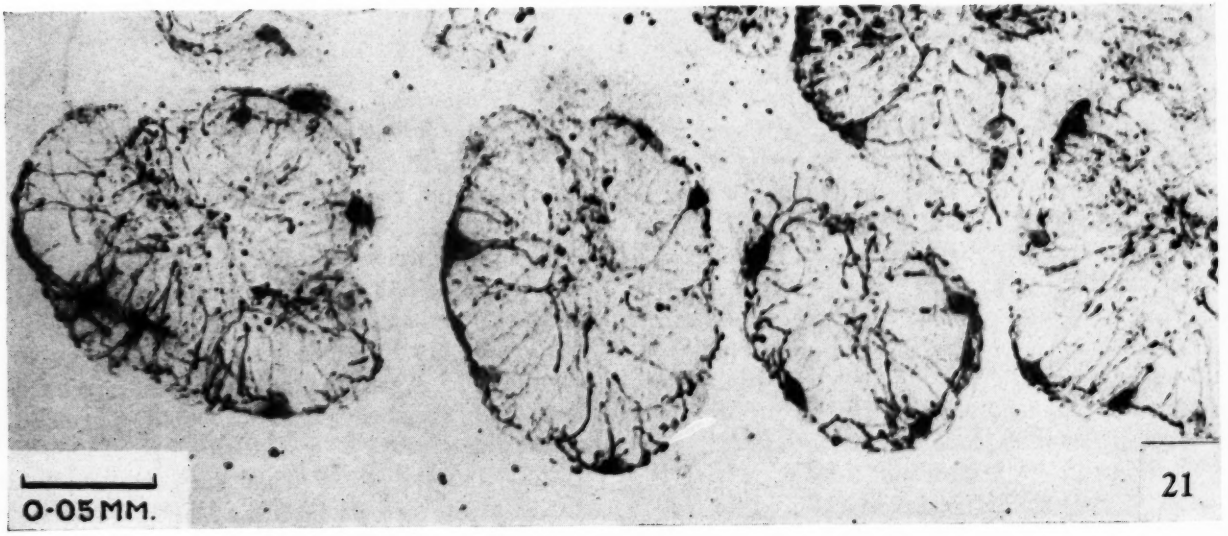
(a) *Nature of the pigment.*—The pigment gives the usual chemical reactions of melanin. It is insoluble in all the ordinary solvents except strong solutions of alkalies, in which it dissolves with some difficulty. It could be slowly bleached by the action of strong sunlight. It does not give the Prussian blue reaction for iron, but is easily impregnated with silver solutions.

(b) *Distribution of the pigment in tumor tissue.*—A naked-eye examination of unstained sections shows that the pigment is unevenly distributed in lobes of tumor tissue (Fig. 4). It is more densely deposited in the peripheral cords and is scantier in the central areas. It occurs as, (I) a fine dust of golden or dark brown particles in the cytoplasm of cells in stratum germinativum and some tumor cells; (II) as diffuse homogenous brown coloring material in the cell body and dendrites of degenerating melanoblasts; (III) as coarse dark brown grains in the dermis or as clumps in the bodies of melanophores, and (IV) as large amorphous masses in cystic spaces in the center of tumor lobules. In the cells of the stratum germinativum it tends to be more closely aggregated in the zone immediately outside the nuclear membrane.

(c) *Pigment-forming cells (Melanoblasts).*—The most interesting feature of these tumors is a proliferation of dendritic cells. The change in the epithelial cells, as one approaches the tumor mass, is accompanied by an alteration in appearance and an increase in number of the dendritic cells (Fig. 19). In this zone the melanoblasts become more numerous and send out a rich brush of long thin processes between the epidermal cells, while still retaining their place in the basal layer of the stratum germinativum abutting against the dermis. These dendritic cells are not easily recognizable by the usual staining methods. They are, however, clearly depicted owing to the presence of a dopa-oxidase in their cell cytoplasm and the fine protoplasmic ramifications, and by their ability to reduce silver salts from solutions. As the expanding epithelial cones fuse and assume the shape of a growing bud the proliferated dendritic cells form a rich protoplasmic network on its dermal surface (Figs. 20 and 21). The dendritic cells move away from the periphery as the epithelial bud grows in mass. They however retain contact with the dermis by a thick process which usually ends in a globular or mushroomed terminal (Figs. 22 and 23). The dendritic cells







FIGS. 19-21

assume an elongated spindle shape on receding from the basal layer. The dendrites become fewer, longer and more filiform as the cells migrate away from the periphery and are caught up in the mass of neoplastic epithelial cells. This migration of dendritic cells has been elegantly described by Masson (11) and by Caudiére (5). These cells appear to be unable to transfer the precursor of the pigment to the adjacent tumor cells, or alternatively the tumor cells lose the capacity of accepting and elaborating the pigment. The pigment, therefore, begins to accumulate in the cell body and branches of the dendritic cells. After their being bereft of their dermal associations, the dendrites thicken and coarsen. Gradually the cells become shrunken and are shorn of most of their branches (Fig. 24). A thick short stump may remain attached to the shrunken, degenerating cells, before they are sloughed off into the amorphous debris. Similar degenerative changes in melanoblasts have been described by Schneider (20) in luetic infiltrations of the epidermis with *Trep. pallidum* and observed by us in a case of a fungus granuloma of the nipple in a 55 year old male.

It is necessary, however, to determine whether the evident increase in the number of dendritic cells at the growing edges of these tumors is genuine or spurious. Rous (18) and Beard (2) have observed that Shope papilloma in rabbits is frequently grey, brown or black with melanin. They noticed (2) that pigmented tumors developed "only where the hair was pigmented . . . When the first epithelial thickening took place, melanoblasts similar to those nearby the unaffected epidermis proliferated in the basal part of the papillomatous epidermis and often became extraordinarily abundant, and black with pigment." They were of the opinion that "pigmented growths arise because these cells (melanoblasts) become involved in the pathological process though not themselves affected by the virus." Masson (12) while discussing the appearances in macular pigmented nevi has expressed the following view. "At first it was attempted to show this excess of branching cells to be the result of their hyperplasia. I do not believe this to be true." He attributes the apparent increase in number of these cells "to an exaggeration of amboceptric differentiation, to the detriment of malpighian differentiation." Caudiére has also expressed the opinion that No part of them [pigmentary cells] shows signs of proliferation. The tumors that do present these characteristics are symbiotic, pigmentary cell epitheliomas." (5). These views deserve most careful consideration, although it must be admitted that the appearances observed in the tumors described above are very suggestive of a true hyperplasia in the spreading zone of the tumor tissue. It is also difficult to accept the opinion of Caudiére that "They are not pigmentary tumors, they are pigmented tumors" (5), as in several cases the lesions start as a deeply pigmented patch, which may later grow discolored towards the central part of the lesion. The pigment-producing cells are actively associated with the growth of neoplastic tissue, although they fail to keep pace with the increase in number of other epithelial cells and are later completely choked by them.

(d) *Accumulation of pigment in tumor tissue.*—The dark color of these tumors is due not only to the presence of pigment particles in the melanoblasts and some tumor cells, but also to a lack of normal elimination of dead epithelial cells. Towards the center of the tumor lobes the keratinized bodies of epithelial cells and the degenerated bodies of melanoblasts are cast off in the debris of necrotic material. The pigment, however, remains unaltered and is retained in cystic spaces and fissures or in the widened follicles involved in the neoplastic process. There appear also groups or circumscribed masses of bulky ovoid or fusiform cells in the superficial layers of the dermis heavily loaded with coarse granules of brown pigment (Fig. 25). They often lie in close proximity to the lobes and cords of tumor tissue and are evidently macrophages which have engorged themselves with pigment. The exact source of pigment in these cells is not very clear. These macrophage cells are never encountered in the body of the neoplastic cords. They are only seen in the connective tissue stroma separating the lobes. These appearances suggest that the macrophages take up formed pigment which is "spilled over" in the dermis and which is not retained by tumor cells. These macrophages have, therefore, been correctly termed melanophores and are distinct in origin

DESCRIPTION OF FIGURES 19 TO 21

FIG. 19.—A composite picture of camera lucida drawings of seven fields, from a biopsy of tissue in case 13. It shows an increase in the number and the alteration in the morphological characters of the melanoblasts in the peripheral zone of the tumor mass. Dopa reaction.

FIGS. 20 and 21.—A rich protoplasmic network of the processes of dendritic cells on the surface of epithelial buds from case 13. Dopa reaction. Fig. 20, Mag. × 200; Fig. 21, Mag. × 375.

and evolution from the melanoblasts described above or the nevus cells encountered in benign or malignant melanomas

II. Regression of Neoplastic Changes

The central area of the lesion in Case 8 showed a tendency towards healing and a replacement of the neoplastic cells by epithelium without evident proliferative activity. Similarly in several centrally located areas in Case 15 there was a disappearance of the Bowenoid change and its replacement by a normal looking stratified squamous epithelium. Such retrogression of experimental tar cancer has been reported. Rous and Kidd (18) observed a raised ulcerated disc after 5 months tarring in one of their rabbits. The growth took on an invasive character during the next 4 months. Later it began to grow smaller and disappeared completely in another 4 weeks. A similar carcinoma with metastases was described by Yamagiwa and Ichikawa, which retrogressed after 630 days of growth. The suggestion therefore that proliferating epithelial buds or Bowenoid changes should be interpreted as a carcinoma could not be accepted without reservations. These conditions should be looked upon as precancerous changes which have the potentiality of developing into a carcinoma with the introduction of other factors which are not so well understood at present.

III. Development of Cancer in the Altered Epidermis

The invasive character of cell proliferation in the central portions of the lesions in Cases 11 and 15 reemphasize the importance of a distinction between tumor inception and tumor formation. The latter condition ensues only when appropriate conditions exist in a area for an infiltrative growth of tumor tissue. This question has been fully discussed previously (9) and need not be entered into again. The importance of a precocious stroma reaction some distance away from the proliferating epithelial buds has been stressed as an influential factor in the limitation of invasive characters in some of the skin cancers by Masson (12) (*stroma reaction précoce*) and it is likely that only when this character fails in the altered dermis, a true carcinoma results.

IV. Spread of Tumors

The spread of these tumors presents certain interesting features. In basal cell carcinomas one is struck with multiple microscopic foci of epithelial proliferation, and the spread along the surface and in depth of the lesion is to a certain extent due to a fusion of these foci and to a growth in mass which results from continued cell division. In the case of the Bowenoid changes in the skin there are two possible methods of lateral spread: (I) an intra-epidermal migration of neoplastic cells as in Paget's disease of the nipple [Muir (14)], or (II) a progressive Bowenoid transformation of normal epithelium in response to an inducing agent continuously being elaborated by active or degenerating neoplastic cells. The latter possibility appears more likely in view of the fact that the line of demarcation between the normal and altered skin is usually very sharp and also because of the arrest of disease after surgical extirpation of affected area of skin. A careful study of the epidermis at the border zone shows few swollen prickle cells just above the normal cells of the basal layer, but no frank neoplastic cells could be detected between normal cells of the malphigian layer as in Paget's disease of the breast. The material at our disposal is inadequate for a solution of this problem, and its elucidation would probably follow the experimental studies now in progress under Cowdry at St. Louis (6).

SUMMARY

1. Fifteen cases of pigmented lesions of the skin which were not melanomas have been reported. Four cases of single pigmented epitheliomas of the skin and four others with multiple lesions have been described.

2. The necessity of a histological diagnosis in all these cases has been emphasized and their relatively benign course has been stressed.

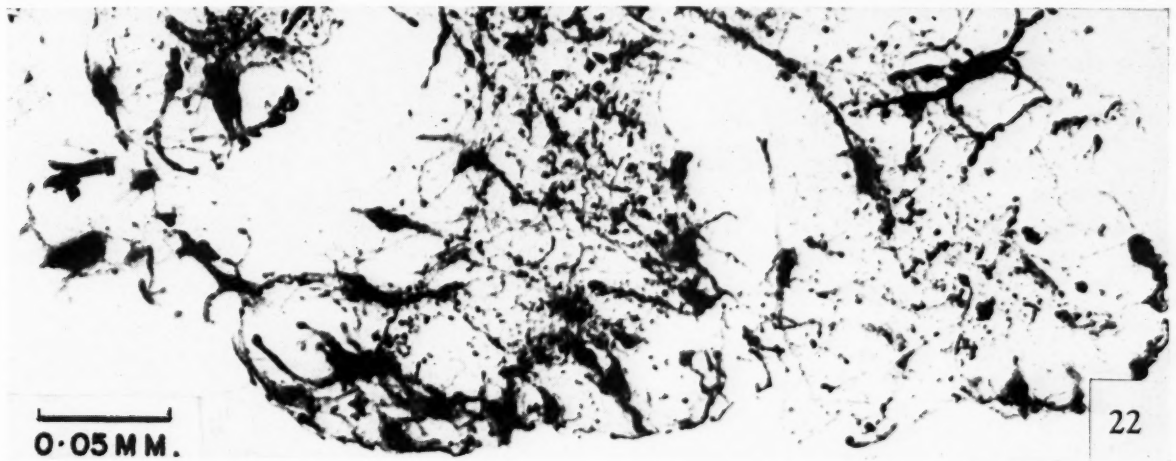
3. The biological nature of these lesions and the role of melanoblasts in their evolution has been discussed.

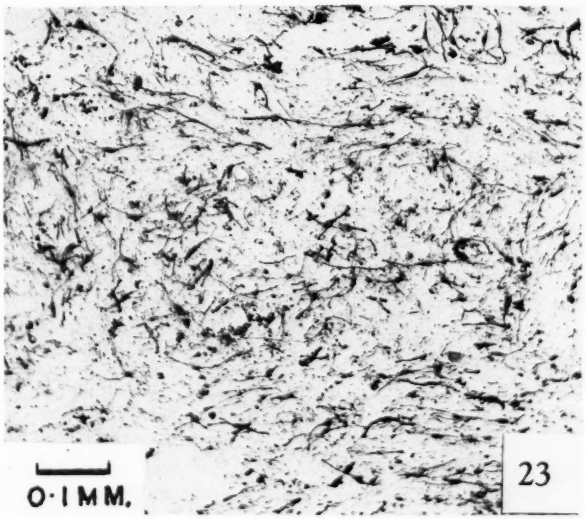
DESCRIPTION OF FIGURES 22 TO 25

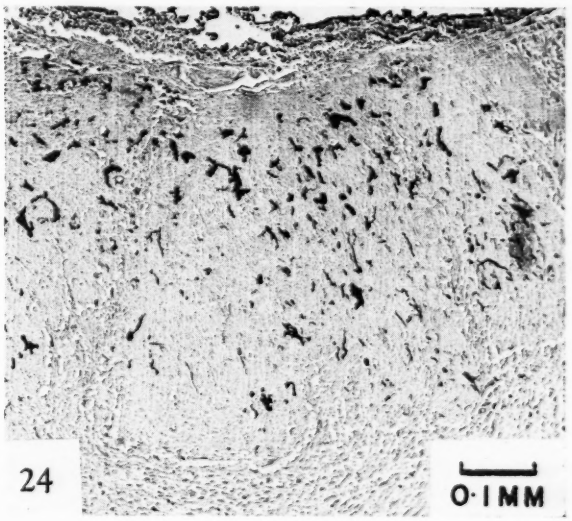
Figs. 22 and 23.—Case 13.: Photomicrographs showing the migration of melanoblasts and the thick processes abutting against the dermis. Dopa reaction. Fig. 22, Mag. × 375; Fig. 23, Mag. × 80.

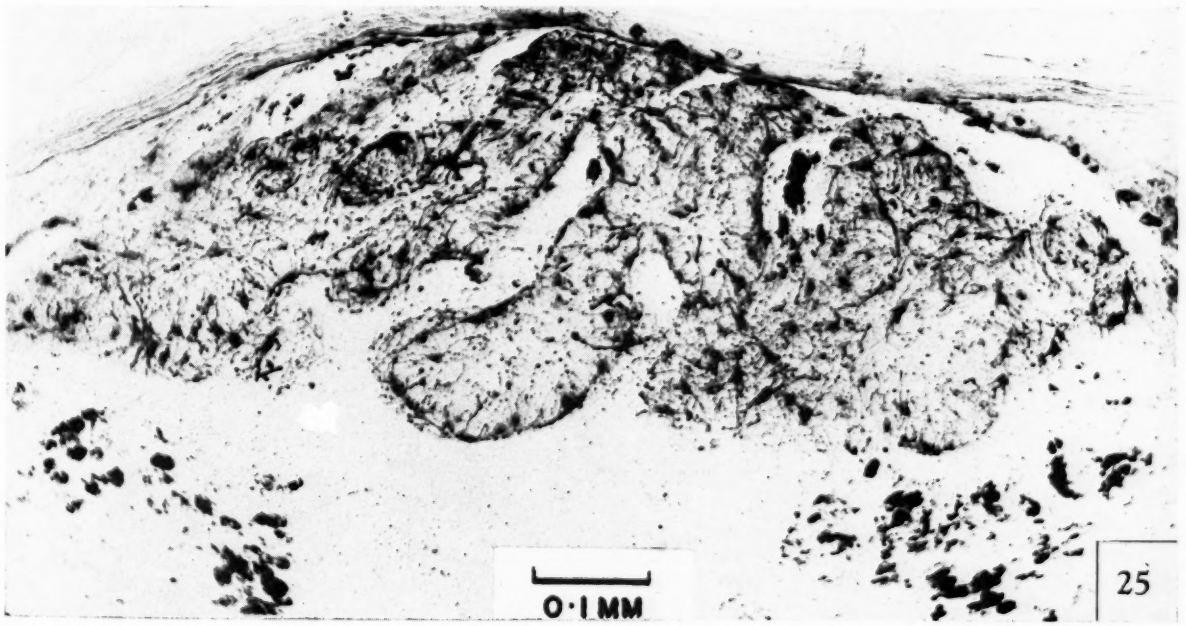
Fig. 24.—Case 12.: Photomicrograph showing the degenerative changes in the migrated melanoblasts, with a shrinkage of cells and loss of dendrites. Silver impregnation. Mag. × 80.

Fig. 25.—Case 13.: A higher power photomicrograph showing the three types of accumulation of pigment in the growing tumor buds, (1) in the debris of necrotic material at the center of tumor lobes (2) in the bodies and process of melanoblasts (3) in groups of melanophores in the dermis. Dopa reaction. Mag. × 150.









FIGS. 22-25

ACKNOWLEDGMENT

It is a pleasure to record the help received from colleagues at this institution and to express an appreciation of the technical ability of the assistants. It would be difficult adequately to express our gratitude to Prof. E. V. Cowdry for much kindness and wise counsel, which has prevented unwarranted conclusions being drawn from the available material.

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Proceedings of Scientific Sessions

HORMONAL IMBALANCES AND TUMORS OF ENDOCRINE GLANDS. W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Tumors of interstitial cells of the testes, pituitary glands, ovaries and adrenal glands have been induced experimentally in animals under conditions of hormonal imbalance produced by the addition of sex hormones, the removal of the sources of intrinsic sex hormones, or the production of excessive gonadotrophins. In most cases genetic factors are of importance in that the tumors are strain-limited. Testicular interstitial cell tumors appear in estrogen-treated mice of the A and JK strains and in their first generation hybrids. The pituitary is assumed to be involved in formation of these tumors. Chromophobe hyperplasias and adenomas occur in estrogen-treated mice of the C57 strain and in their hybrids but rarely in mice of other strains or hybrid groups. The simultaneous administration of androgen partially inhibits their appearance. Whether hormonal mechanisms prevent their appearance in the mice of the resistant strains is not known. Ovarian tumors occur in intrasplenic transplants of ovaries. Under such conditions the ovaries are exposed to excessive intrinsic gonadotrophin, presumably follicle-stimulating hormone, although sex differences exist. The ovarian tumors appearing in roentgen irradiated mice may be explained on a humoral imbalance basis. Adrenal cortical tumors also appear in mice (Woolley) subsequent to gonadectomy at birth or even when older (Gardner). These tumors as well as the testicular interstitial cell and ovarian tumors mentioned above produce physiologically active substances. These tumors will be discussed from some of their genetic and hormonal interrelationships.

COMPARISON OF THE CARCINOGENIC ACTIVITY IN EXTRACTS OF HUMAN LIVER AND OTHER HUMAN AND ANIMAL ORGANS. PAUL E. STEINER, D. WARREN STANGER, and MIRIAM BOLYARD. (Department of Pathology, University of Chicago, Chicago, Ill.)

Ethylene dichloride extracts after saponification were prepared from pooled human livers, kidneys, spleens, hearts and colons. The extracts were made in duplicate from cancer-bearing and noncancer-bearing patients. Similar extracts were made from pooled livers of stillborn infants, swine livers, bovine livers, and swine hearts. The extracts were tested for carcinogenic activity by subcutaneous injection into 1,044 mice of C57 black, A, or our albino strains. The percentage yield of sarcomas at the site of injection in C57 black mice surviving for 6 months was: Noncancerous livers, 58.7; cancerous livers, 14.8; cancerous spleens, 10.4; livers of stillborn infants, 8.1; swine livers tested in strain A mice, 7.5. The other extracts were essentially noncarcinogenic.

THE LOCALIZATION OF STEROIDS IN NORMAL AND CANCEROUS TISSUES BY THE USE OF RADIOACTIVE ISOTOPES AND HISTOCHEMICAL METHODS. S. ALBERT, J. COHEN, R. D. H. HEARD, and C. P. LeBLOND. (Departments of Anatomy and Biochemistry, McGill University, Montreal, Canada.)

By using fuchsin-sulphurous-acid and 2,4-dinitrophenylhydrazine, two reagents supposedly specific for ketosteroids, it can be shown that the histochemical reactions thus obtained in tissues are not suppressed by the removal of ketosteroid-producing organs. In normal and cancerous animals the most intense reactions are found in the ovary, testis, adrenal and accessory sex organs, with little or no reaction in cancerous tissue. It is concluded that these reactions reveal the presence of a non-ketosteroid substance, probably an acetal phosphatide of the plasmalogen family, which may be linked with steroid metabolism.

Using α-estradiol iodinated with radioactive iodine, it was possible to follow the distribution of this compound in the tissues of cancerous mice by means of the Geiger counter. It was found that after 10 hours the largest concentration of this compound occurred in the gastro-intestinal tract, feces and urine, while only minute amounts occurred in the genital organs, accessory sex organs and cancerous tissues.

A PHYSIOLOGICAL MEASURE OF HOST-TUMOR RELATIONSHIP AS SHOWN BY A TRANSPLANTABLE MOUSE RETICULOENDOTHELIOMA. ARTHUR M. CLOUDMAN. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

Measurable physiological changes have been produced by parabiotic operations in mice normally refractory to the transplantable reticuloendothelioma, C198. This leaden strain tumor always involves the liver of susceptible leaden mice that receive subcutaneous implants of tumor tissue. All other pure strains of mice are resistant to this tumor when the usual technics are employed in tumor tissue transfer.

The transfer of some substance or substances through the medium of body tissues and fluids not normally introduced frequently make refractory C57 black strain mice serve as a successful host for tumor C198. After the altered host has progressively grown the tumor for a certain time interval and the mass has become sizeable the tumor itself undergoes a physiological change. After this the tumor can be easily transferred to members of the C57 black strain. However, it will still grow in the leaden mice. Furthermore, whatever tumor change was induced by growth in a black mouse is weakened or lost by growth for one tissue transfer generation in a leaden mouse.

The data presented reveal that (a) parabiosis alters refractory C57 black strain mice, making many of them susceptible to implants of tumor C198; (b) the altered host can change the implanted tumor; (c) this changed tumor can be successfully transferred to other C57 black mice; and (d) C57 black mice growing the altered C198 tumor remain resistant to unaltered C198 taken directly from a leaden donor mouse.

THE NEOPLASTIC TRANSFORMATION OF GRANULOSA CELLS IN GRAFTS OF NORMAL OVARIES INTO SPLEENS OF GONADECTOMIZED MICE. J. FURTH, and H. SOBEL. (Department of Pathology, Cornell University Medical College, New York, N. Y.)

Growth of granulosa cells were produced in 29 (67 per cent) of 43 mice by grafting fragments of normal ovaries into the spleens of gonadectomized mice as described by Biskind and Biskind. After intrasplenic subpassages into gonadectomized mice, the splenic growth in one of these mice became transformed into a neoplasm readily transplantable into the subcutaneous tissue of normal mice and occasionally metastasizing to the lung (Strain B1). A second transplantable strain (B2) was derived from another mouse that had a splenic growth of granulosa cells with secondary nodules in the liver. This growth proved readily transplantable in the spleen, from which it frequently metastasized to the liver, but not in the subcutaneous tissue. The secondary changes in mice bearing these 2 transplantable tumors indicate the discharge of estrogens by the tumor cells. The blood volume of mice bearing subcutaneous tumors of these strains is elevated and their livers show cavernous congestion characteristic of hypervolemia. These experiments serve to illustrate how hyperplasia of normal cells can lead to neoplasia and enable an analysis of the factors bringing about this transformation.

FURTHER STUDIES ON THE PATHOGENESIS OF THE OVARIAN TUMORS IN MICE. M. H. LI, and W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Our previous experiments demonstrated that a pituitary-gonadal endocrine imbalance may be induced by the intrasplenic transplantation of ovaries in castrated male and female mice and that the imbalances may result in the formation of granulosa cell tumors, luteomas, or mixed cell tumors.

Most extensive studies have been made by using inbred mice of the A, C3H, and C57 strains, and several groups of hybrid mice (A × C3H and CBA × C57). Ovarian tumors have appeared in intrasplenic transplants in mice of the A, C3H and C57 strains and in hybrids; there is apparently no strain limitation in the development of ovarian tumors in intrasplenic autotransplants or homotransplants of ovaries in castrated mice. Ovarian tumors have not been observed in the intrasplenic ovarian transplants in unilaterally gonadectomized male and female mice. The formation of tumors in the intrasplenic ovarian transplants was prevented by weekly administration of small doses of α-estradiol benzoate or testosterone propionate. Similar treatment of progesterone, however, was not effective. Daily injection of a gonadotrophic hormone from pregnant mare serum for short periods exerts a stimulating effect on the growth of the transplants and on tumor formation. These observations are interpreted to substantiate further the assumption that overaction of gonadotrophic hormones is responsible for the genesis of the ovarian tumors in mice.

CORRELATION OF A BIOLOGICAL TEST WITH CLINICAL DIAGNOSIS IN HUMAN MALIGNANCY. HOWARD H. BEARD, SAMUEL L. LIBERT, and B. HALPERIN (by invitation). (Department of Physiological Chemistry, The Chicago Medical School, Chicago, Ill.)

Forty known malignant urines were extracted with an equal volume of alcohol and ether for 2 days in the small Koch extractor. The process was then repeated for another 2 days. Solvents were combined and evaporated under reduced pressure and the water residue diluted so that 2 cc. represented 100 cc. of the original urine. This amount was injected intraperitoneally into immature white rats and the animals were sacrificed from 1 to 4 days later. Litter mates of the same sex and approximate body weight were used as controls without injection. The gonads, spleen and body weight were made soon after death by an overdose of nembutal. The body weight/gonad and body weight/spleen ratios were then calculated for all the animals. In 39 of the 40 known malignant urines these ratios decreased from 20 to 80 per cent and this observation constituted the biological test of malignancy. Nonmalignant urines and those from normal individuals gave ratios that decreased from the control ratios by less than 15 per cent and were considered negative. The average degree of hypertrophy observed was as follows: spleen, 483 to 673 mgm. (39 per cent); male gonads, 1283 to 1991 mgm. (55 per cent), and female gonads, 219 to 365 mgm. (72 per cent). Histological studies showed an intense passive hyperemia of the spleen and intense spermatogenesis in the testes. The female gonads were not sectioned. These results are in agreement with those of Roffo, and Krebs and Gurchot. It is concluded that *all* malignant urines so far tested contain a cancer hormone (probably of sterol nature) which is the cause of the biological test described above. We believe

that this hormone acts through the pituitary to produce increased amounts of a gonadotrophic hormone which is the immediate cause of the hypertrophy of the spleen and gonads. We are not yet convinced that this is an Ascheim-Zondek test but further work may prove this to be so.

THE EXCRETION IN THE URINE OF METABOLITES OF ADRENAL CORTICAL HORMONES IN HEALTH AND DISEASE, INCLUDING NEOPLASTIC GROWTH. KONRAD DOBRINER, S. LIEBERMAN, and C. P. RHOADS. (Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

Two metabolites of adrenal cortical hormones, androstanediol-3α, 11β-one-17 (Mason and Kepler: *J. Biol. Chem.*, **161**:235. 1945) and etiocholanol-3α-dione-11, 17 (Lieberman and Dobriner: *J. Biol. Chem.*, **166**:773. 1946), have already been isolated from the urine of normal and diseased persons. The purpose of the present communication is to report the isolation from urine of another steroid of adrenal cortical origin, Δ 9-11 etiocholenol-3α-one-17, which is probably a dehydration product of etiocholandiol-3α, 11β-one-17.

Attention is called to the fact that this newly isolated compound has been found quite commonly, although not universally, in the urine of patients with disease states: cancer, lymphatic leukemia, hypertension and adrenal cortical disorders of the Cushing's syndrome type, but has been found in the urine of only 2 of 23 normal subjects. It is suggested that this compound is a product of deranged metabolism of adrenal cortical hormones or a metabolite of an abnormal precursor.

EFFECT OF DIET DEFICIENT IN CERTAIN AMINO ACIDS ON THE INDUCTION OF LEUKEMIA IN dba MICE. JULIUS WHITE, FLORENCE R. WHITE, and G. B. MIDER. (National Cancer Institute, Bethesda, Md., and Department of Surgery, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

A comparative study was made of the restriction of cystine, lysine and tryptophane, respectively, on methylcholanthrene-induced leukemia in strain dba mice. Each of the diets employed was so restricted in one of the foregoing amino acids that growth of young mice was prohibited, but indefinite maintenance was possible. The same diets, each supplemented by the amino acid in which it was deficient, permitted good growth. There was no significant decrease in the incidence of leukemia among the mice on diets restricted in either lysine or tryptophane. There was a reduction in the incidence of leukemia from 92.1 per cent for the control group to 55 per cent in the group of mice whose diet was restricted in cystine. The data indicate that under the conditions of the experiment, cystine played a role in the development of leukemia not associated with its properties as an essential amino acid for growth but some other attribute not yet determined.

INFLUENCE OF ESTROGENS AND ANDROGENS ON DEVELOPMENT OF DIETARY CIRRHOSIS IN RATS. IRA T. NATHANSON and PAUL C. ZAMECNIK. (Medical Laboratories, Collis P. Huntington Memorial Hospital, Harvard University; and Tumor Clinic, Massachusetts General Hospital, Boston, Mass.)

Ninety-nine 6 week old female and forty-eight 8 month old male Sprague-Dawley rats were divided into groups as follows: (1) control diet, (2) control diet plus androgen, (3) control diet plus estrogen, (4) modified Gyorgy diet, (5) modified Gyorgy diet plus androgen and (6) modified Gyorgy diet plus estrogen. 2.5 mgm. of testosterone propionate and 0.2 mgm. of estradiol dipropionate were injected subcutaneously into the appropriate groups twice a week for 4 months. Animals from all groups were sacrificed at intervals, and the experiment terminated at 8 months. There was marked fatty infiltration in groups 4 and 5. Group 6 animals, however, showed little fatty infiltration, particularly in the male rats, indicating a striking lipotropic effect of estrogen.

The series of the female rats on the experimental diet showed both gross and microscopic evidence of cirrhosis. Androgens appeared to aggravate the cirrhosis, while estrogens appeared to have an ameliorating effect.

EFFECT OF VARYING THE PROTEIN (CASEIN) CONTENT OF THE DIET ON THE FORMATION OF TUMORS IN THE MOUSE. ALBERT TANNENBAUM and HERBERT SILVERSTONE. (Department of Cancer Research, Michael Reese Hospital, Chicago, Ill.)

Since it is generally believed that protein metabolism may play an important role in the formation of tumors, the effect of different levels of dietary protein (casein) was studied. "Synthetic" diets were utilized and protein levels of 9, 18, 27, 36 and 45 per cent were obtained by substituting casein for cornstarch. All other components of the diets were left unchanged. The modifying effect of the level of protein was evaluated with the carcinogen-induced skin tumor, the spontaneous mammary carcinoma, and the spontaneous hepatoma of the mouse.

No significant effect on either the incidence of induced skin tumors or their average time of appearance was observed. With the spontaneous mammary carcinoma, no difference in incidence was found but the tumors may have appeared somewhat earlier, on the average, in the group being fed 18 per cent casein. In the three groups of C3H male mice receiving diets containing 9, 18, and 45 per cent casein, the percentage of spontaneous hepatomas at 13 months were 11, 61, and 38 respectively, indicating that the "low" and "high" protein diets led to fewer tumors than the diet with "moderate" protein.

It may be concluded that varying the protein (casein) content of the diets, within the limits as indicated, probably has little effect on the formation of many types of tumors, but may have a significant effect on certain special kinds.

DIFFERENCE IN ACTIVATION OF PROTEOLYTIC ENZYMES IN NORMAL LIVER AND HEPATOMA, AS DETERMINED BY MEANS OF A NEW MONOMETRIC METHOD FOR FOLLOWING PEPTIDE CLEAVAGES. PAUL C. ZAMECNIK and MARY L. STEPHENSON. (Medical Laboratories, Collis P. Huntington Memorial Hospital; and Tumor Clinic, Massachusetts General Hospital, Boston, Mass.)

A manometric method has been devised, which facilitates the study of reaction kinetics involved in the hydrolysis of tyrosine-containing peptides by catheptic enzymes. This method depends on the inclusion in the reaction mixture of a bacterial decarboxylase, which liberates carbon dioxide from *l*-tyrosine as the latter is split from peptide linkage. Since the decarboxylase is present in excess, the rate of carbon dioxide production from tyrosine reflects the rate of the peptide cleavage. This method makes it possible to follow in detail the activation mechanism of the catheptic enzyme.

Ultrafiltrates have been prepared from normal rat livers, and from primary hepatomas induced by butter yellow. The ultrafiltrates of normal livers and of the nonmalignant portion of the hepatoma-containing livers activate a purified catheptic enzyme more than ultrafiltrates prepared from hepatoma nodules.

THE INHIBITING ACTION OF AMORPHOUS AND CRYSTALLINE PENICILLIN AND STREPTOMYCIN PREPARATIONS ON THE METABOLISM OF TUMORS AND OTHER TISSUES. DEAN BURK, MARIE L. HESSELBACH, and CLARA E. FISCHER. (National Cancer Institute, Bethesda, Md.)

Amorphous preparations of penicillin have been found to produce a marked inhibition of respiration of tumors and normal tissues (*e.g.*, spontaneous breast adenocarcinoma, transplanted Barrett C3HBA adenocarcinoma, Earle L sarcoma, kidney, spleen, and liver of mice). The inhibition is immediate (detectable manometrically within a few minutes) and progressive, attaining practical completion (95 to 100 per cent) within one to several hours, depending upon the amorphous preparation and concentration employed (range, 0.1 to 10 mgm./cc.). Penicillin G several-times recrystallized (1,660 Oxford units/mgm.) was approximately one-tenth as inhibitory on a weight basis as several amorphous preparations assaying 1,000 to 1,500 Oxford penicillin units/mgm. Whether this small activity is due to the crystalline penicillin itself, or to possible traces of the "amorphous factor" still extant as impurity, remains to be determined. Crystalline streptomycin salt was still less inhibitory on a weight basis.

Treatment of various amorphous preparations with *B. subtilis* penicillinase, to remove essentially all penicillin activity against microorganisms, reduced the respiration inhibiting activity per mgm. by 25 to 75 per cent, depending upon the ratio of the amorphous factor to penicillin in the preparation. The amorphous factor can thus act on respiration independently of the presence of penicillin. Synergistic action (as occurs in the case of the enhancement factor of Welch, Randall, and Price) has not been definitely indicated. In any event, metabolic analysis offers a rapid and comparatively sensitive method of assay. Tumor glycolysis was nearly as subject to inhibition by the amorphous factor as was respiration.

EFFECTS OF AN ASCORBIC ACID DEFICIENCY ON TUMORS. WILLIAM v. B. ROBERTSON, A. J. DALTON, and WALTER HESTON. (National Cancer Institute, Bethesda, Md.)

The effect of an ascorbic acid (vitamin C) deficiency on tumors was studied on transplants of a fibrosarcoma (N.C.I. – C – 2663) in an inbred family of guinea pigs. Animals were maintained on an adequate diet until the tumor transplants became palpable, and then were placed on the scorbutigenic diet.

After the guinea pigs had been fed the vitamin C-free diet for 2 weeks, the tumors appeared to become attached to the skin and belly wall, whereas transplants in animals on an adequate diet were loose and easily movable. At necropsy, the scorbutic guinea pigs were found to have large amounts of hemorrhagic connective tissue connecting the tumor capsule with the deeper layers of epidermis and with the musculature of the body wall.

Transplants of this fibrosarcoma show a core of central necrosis surrounded by a margin of healthy tumor tissue, but the tumors in scorbutic hosts showed not only much larger areas of central necrosis but also many areas of focal necrosis scattered throughout the periphery.

The ascorbic acid concentration of the tumors in the scorbutic cavies was essentially zero; that of the nonneoplastic tissues, although considerably below normal, was still appreciable.

The collagen concentration of tumors in the scorbutic animals averaged 3.7 per cent, as compared with the concentration of 8.9 per cent found in the tumors from normally fed controls.

The rate of tumor growth as measured by external calipering was the same in the scorbutic and control groups for a fortnight, after which the tumors in scorbutic guinea pigs grew much more slowly. The average weight of tumors removed from 14 scorbutic and moribund animals was 7.6 gm., whereas tumors taken concurrently from 7 normal animals had an average weight of 29.6 gm. This difference was found to be statistically significant ($p < 0.01$).

DESAMIDATION OF GLUTAMINE AND ASPARAGINE IN NORMAL AND NEOPLASTIC HEPATIC TISSUES. MAURICE ERRERA (by invitation) and JESSE P. GREENSTEIN. (National Cancer Institute, Bethesda, Md.)

Fetal rat liver possesses little or no asparaginase activity but does possess a high glutaminase activity. In adult rat liver, the relative activity of these enzymes is reversed, the glutaminase activity being extremely weak and the asparaginase activity very high. When the adult liver becomes neoplastic, the fetal pattern is noted in the hepatoma, *i.e.*, a near-disappearance of asparaginase activity and concomitant rise in glutaminase activity. That a tumor may possess the metabolic characteristics

of the corresponding embryonic form is not surprising by now.

The rate of desamidation of glutamine and asparagine in homogenates of all three kinds of hepatic tissues is greatly increased by added pyruvate. The pyruvate is not consumed in the reaction, but plays the role of a cosubstrate. This effect of pyruvate is almost exclusively a property of the liver, and is not observed to any great extent in other normal tissues. The fact that it is noted in hepatomas but not in any other tumors of different histogenesis shows that in this respect the hepatoma bears the imprint of its tissue of origin, and suggests a chemical method of distinguishing hepatomas from other kinds of tumors.

COBALT INHIBITION OF TUMOR RESPIRATION AND PROTECTION BY HISTIDINE. JOHN HEARON, ARTHUR L. SCHADE, HILTON LEVY, and DEAN BURK. (National Cancer Institute, Bethesda, Md., and Overly Biochemical Research Foundation, New York, N. Y.)

Cobalt has been shown previously to inhibit tissue respiration at concentrations of approximately 5 to 50 p.p.m. The inhibition is progressive with time, and tumor tissue is particularly sensitive. It has been found that the inhibition of tumor respiration may be prevented by additions of histidine at a molar ratio of histidine to cobaltous ion of 2 to 1 or greater. This protection is explicable on the basis of the reversible formation of a 2:1 histidine-cobalt complex, cobaltodihistidine, whereby the equilibrium constant, $K = (\text{cobaltodihistidine})\ (H+)^2/(\text{histidine})^2\ (Co++) = 7.5 \times 10^{-7}$ at 38° C. The degree of protection afforded by a given concentration of histidine at any level of cobalt concentration may be correlated with the degree of completion of the reaction, and the observed inhibition in the presence of histidine is in accord with the concentration of free cobaltous ion calculated from the equilibrium expression. It may be concluded that the cobaltodihistidine is nontoxic. The rather slight protection given by other alpha amino acids and histamine parallels the observed lower coordination affinities of the compounds for cobaltous ion, except in the case of cysteine which forms a 3 cysteine: 1 cobaltous complex of high affinity that is rapidly oxidized to the cobaltic state and affords considerable protection. The progressive inhibition of tumor respiration by cobalt can only be halted but not reversed by additions of histidine. Thus, it appears that the combination of cobalt with the tissue component concerned is essentially irreversible, even in the presence of histidine. Further studies on the mechanism are in progress.

PURIFICATION AND PROPERTIES OF DEHYDROPEPTIDASES FROM NEOPLASTIC AND NORMAL TISSUES. JOSEPH SHACK. (National Cancer Institute, Bethesda, Md.)

Previous investigators have postulated from tissue distribution studies the existence of a dehydropeptidase I splitting glycyldehydroalanine with a uniformly high activity in tumors and of a dehydropeptidase II splitting chloracetyldehydroalanine and absent in tumors.

A purification and study of these enzymes from rat tissues has been carried out. By differential centrifugation at 3,000 and 18,000 R.P.M., it was found that the bulk of dehydropeptidase I of kidney is firmly bound to particulates sedimentable only at high speeds. In contrast the dehydropeptidase I of liver and tumor and the dehydropeptidase II of liver and kidney remain in the supernant. A hundred-fold concentration of dehydropeptidase I free of dehydropeptidase II has been achieved by differential centrifugation, enzymatic digestion and salt fractionation of kidney extract. The soluble enzymes have been purified by low temperature alcohol fractionation. The separation of activities made in the fractionation procedures confirm the existence of 2 distinct enzymes. They have been compared with respect to pH dependence, kinetics and specific inhibition. Neither is inhibited by azide, iodide or fluoride. Both are inhibited by cyanide and thioglycolate, inhibitions reversible on dialysis. Iodoacetate inhibits dehydropeptidase II (also reversed on dialysis) but has no effect on dehydropeptidase I.

These results indicate that the absence of dehydropeptidase II activity in hepatoma is due not to a change in specificity but to the disappearance of the enzyme as a result of malignancy. Comparative studies of purified dehydropeptidase I from liver and hepatoma have shown no differences in catalytic properties.

PHOSPHORYLATED INTERMEDIATES IN TUMOR GLYCOLYSIS. G. A. LePAGE. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The rate of glycolysis has been reported to be high in tumors. There has been considerable controversy as to whether this was a phosphorylative or non-phosphorylative glycolysis. While data available can all be reasonably explained on the basis of a phosphorylative glycolysis and the enzymes necessary all appear to be present in tumors, the question of what type of glycolysis is operative had not been conclusively settled.

In this investigation analyses were made, using methods established as adequate for other tissues, for the intermediates of the Meyerhof phosphorylative glycolysis system. Tissues were fixed in liquid air. Components analyzed for included inorganic phosphorus, the adenine nucleotides, phosphocreatine, glycogen, glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, hexosediphosphate, phosphoglyceric acid, phosphopyruvic acid and lactic acid. Analyses were confirmed in certain cases by isolation in high yield. Tumors so studied included several transplantable and certain primary rat and mouse tumors, and human carcinoma.

Glycogen was found to be low except in the human tumor samples. Lactic acid was elevated several fold above that of differentiated tissues in all cases. The levels of other intermediates conformed with those found in differentiated tissues. Modification of the physiological state of certain tumors by production of anoxia or hyperglycemia gave changes which were interpretable on the basis of a phosphorylative system.

THE DPN-CYTOCHROME REDUCTASE CONTENT OF CANCER TISSUE. M. RHIAN and VAN R. POTTER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The enzyme that has been referred to as DPN-cytochrome reductase catalyzes the reaction between diphosphopyridine nucleotide (DPN, coenzyme I, cozymase) and cytochrome *c*. When this enzyme is functioning, the substrate-hydrogen from the DPN-linked dehydrogenases is transported to oxygen via the cytochrome system.

Using the malic dehydrogenase system as a source of reduced DPN it has been possible to devise an assay system for the determination of DPN-cytochrome reductase. Assays have been carried out on normal rat liver, heart, kidney and brain tissue; and Walker carcinoma 256, Jensen sarcoma, Flexner-Jobling carcinoma and primary hepatomas, all from rats. It has been shown that the cancer tissues are extremely low in this enzyme as compared with the normal tissues studied thus far.

The results can be interpreted in terms of the balance between the glycolytic and the oxidative enzymes, since a relative deficiency of this enzyme would diminish the rate of oxidation of reduced DPN by oxygen, leaving it to be oxidized by pyruvate, which in turn would be converted to lactate. Thus a deficiency in cytochrome reductase could result in the glycolytic type of metabolism that is found in tumors.

SUCCINOXIDASE STUDIES OF THE LIVER CELLS OF MICE FED CARBON TETRACHLORIDE. KRETCHMER, N. (by invitation), TSUBOI, K. K. (by invitation), and BARNUM, C. P. (Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.)

Alterations in the succinoxidase system that took place during the period of carbon tetrachloride feeding were presented from studies on cytoplasmic fractions of the mouse liver cell.

Liver tumors were induced in C3H mice over a period of 200 days by feeding 0.1 cc. of 40 per cent carbon tetrachloride in olive oil every 4 days. Animals were sacrificed at intervals for the succinic oxidase assay. The assays were conducted on the cytoplasmic particulates obtained by methods of differential centrifugation.

During carbon tetrachloride poisoning of the mouse liver, there is initially a decrease in the cytoplasmic succinoxidase activity of the liver cell; as the induction proceeds, the enzyme activity reaches normal values and exceeds the normal in the 60, 75, and 90 day periods. After 90 days the enzyme activity follows a downward trend such that at 200 days and at tumor, the enzyme activity is somewhat below the normal.

THE INTERFACIAL DENATURATION OF PROTEINS IN THE PRESENCE OF AROMATIC DIAMIDINES AND NUCLEIC ACIDS. M. J. KOPAC. (Department of Biology, Washington Square College of Arts and Sciences, New York University, New York, N. Y.)

These experiments augment the work reported at the A. A. A. S.-Gibson Island Conference of 1946 (*Cancer Research* 7:44-46. 1947). The effects of stilbamidine, propamidine, and *bis*-amidinomethyldibenzyl on the denaturation of bovine plasma albumin and of crystalline ribonuclease (Kunitz) at oil-water interfaces were measured.

The interfacial denaturation of albumin (2 mgm./ml.) was enhanced by stilbamidine (0.001M), less so by propamidine (0.001M), and completely inhibited by *bis*-amidinomethyldibenzyl (0.001M). With an albumin concentration of 5 mgm./ml., or higher, only stilbamidine enhanced surface denaturation, whereas others depressed it.

On adding Na zymonucleate (1 mgm./ml.) to the albumin-diamidine preparations, the diamidines were nearly completely antagonized. The combination of stilbamidine + propamidine, each at 0.001M, produced a typical stilbamidine effect. On adding Na zymonucleate (1 mgm./ml.) to this preparation, the action of stilbamidine was abolished and a typical propamidine effect was elicited, indicating that stilbamidine was preferentially bound by the nucleic acid. Stilbamidine was partly neutralized by yeast adenylic acid (1 mgm./ml.).

The interfacial denaturation of crystalline ribonuclease (1 mgm./ml.) was strikingly enhanced by stilbamidine (0.001M) and by propamidine (0.001M) and completely prevented by *bis*-amidinomethyldibenzyl (0.001M). All diamidine effects were abolished on addition of Na zymonucleate (1 mgm./ml.). Sodium thymonucleate (1 mgm./ml.) was less active than Na zymonucleate in abolishing the stilbamidine effect. Stilbamidine was not neutralized, however, if the Na zymonucleate was previously incubated with ribonuclease for 2 to 4 hours. Following incubation of Na thymonucleate with ribonuclease, stilbamidine was considerably neutralized.

These data indicate that certain diamidines enhance interfacial denaturation because they weaken side-chain linkages in protein molecules. No appreciable increase in interfacial denaturation was observed if these diamidines were removed before exposing the proteins to interfacial forces. The increased denaturation, therefore, results from the simultaneous action of surface forces with the diamidines.

Stilbamidine in the presence of other diamidines was preferentially bound by nucleic acids. These data may explain why stilbamidine produced the drastic action on the nucleoproteins tested to date. This compound, a denaturating adjuvent, is readily bound by nucleic acids.

LARGE SCALE PREPARATION OF THE TUMOR-NECROTIZING POLYSACCHARIDE FROM *S. MARCESCENS*. ADRIAN PERRAULT and M. J. SHEAR. (National Cancer Institute, Bethesda, Md.)

The cultivation of strain 724 of *S. marcescens* in a synthetic medium is being carried out in lots of up to 350 l. each. The organisms are separated, and the active agent in the filtrates concentrated to 1/200 of the original volume.

After further concentration and purification, bioassays for potency are carried out in mice bearing sarcoma 37. The potency of the final product is similar to that obtained in the small-scale preparations of the active polysaccharide previously obtained with culture filtrates from the "G. W." strain.

Active fractions have been obtained from the organisms themselves, and these fractions are being subjected to further purification. Yields are now measured in grams.

TUMOR NECROTIZING BACTERIAL POLYSACCHARIDE TAGGED WITH RADIOACTIVE IODINE.

ARNOLD M. SELIGMAN, JOSEPH LEITER, BENJAMIN SWEET, and M. J. SHEAR. (Surgical Research Department, Beth Israel Hospital, and Department of Surgery, Harvard Medical School, Boston, Mass., and the National Cancer Institute, Bethesda, Md.)

The polysaccharide from *Serratia marcescens*, which produces necrosis in tumors, was tagged with radioactive iodine (I^{131}). Unattached iodine was removed by dialysis.

Free iodine.—Iodination of 2.5 mgm. of polysaccharide with 0.25 mgm. of iodine resulted in the incorporation of 3 per cent of the iodine or 180 atoms of iodine per molecule of polysaccharide (approximate molecular weight 8,000,000). Ethylene linkages presumably were involved in the reaction. Some loss in tumor necrotizing potency was observed in mice bearing sarcoma 37.

Sodium hypoiodite.—Iodination of 2.5 mgm. of polysaccharide (P_3R) with iodine in the presence of sodium carbonate (10 mgm.) resulted in incorporation of the iodine. When 1.25 mgm., 0.25 mgm., and 0.05 mgm. of iodine were used, 0.7 per cent, 3.5 per cent, and 14.4 per cent of the iodine respectively were attached; the number of atoms of iodine per molecule of polysaccharide attached was 226, 223, and 183 respectively. The polysaccharide molecule, therefore, was readily saturated within a wide range of iodine concentration. Hydrogen, alpha to a carbonyl group, presumably was replaced in this reaction. No loss in tumor-necrotizing properties was observed in mice with sarcoma 37.

Mandler candle (1 in. long) filtration of a solution of tagged polysaccharide (25μgm. per ml.) resulted in a 20 per cent loss of radioactivity.

Blood disappearance curves in mice, rabbits and man showed 40 per cent loss of radioactivity in 10 minutes and 75 per cent loss in 30 to 60 minutes.

The ratios of the radioactivity of tissues to that of circulating blood, when normal mice were sacrificed 1 hour after the injection of 25μgm. of iodo-polysaccharide were, liver 1.3; lung 0.44; kidney 0.30; and thyroid 0.30. The ratios of the radioactivity of liver and tumor to that of circulating blood, when mice bearing sarcoma 37 were sacrificed after injection of 12.5 μgm. of iodo-polysaccharide, were as follows:

	Liver	Tumor
At 1 hour	2.8	0.23
At 14 hours	1.4	0.22
At 24 hours	4.9	0.43

SOME EFFECTS OF IODINATED BACTERIAL POLYSACCHARIDE ON PATIENTS WITH MALIGNANT TUMORS.

THEODORE SACK, and ARNOLD M. SELIGMAN. (Surgical Research Department, Beth Israel Hospital, and Department of Surgery, Harvard Medical School, Boston, Mass.)

Iodinated tumor-necrotizing polysaccharide from *S. marcescens* was administered intravenously to 7 patients with metastatic carcinoma. Individual doses varied between 2 and 3,000 μgm., repeated doses were given at no less than 24 hour intervals, and the maximum total dose to a single patient was 3,405 μgm. With some individual variations the post-injection reactions followed a common pattern, namely, a chill, with a subsequent temperature rise sustained for 1 or more hours, then a gradual fall to normal 12 to 30 hours after injection.

The blood pressure uniformly rose during the chill phase, began to fall when the temperature was maximum, and reached minimum levels between 8 and 15 hours after injection. Vigorous anti-shock therapy was necessary in 4 instances. Pulse and respiratory rates roughly followed the temperature curve.

The symptoms were those of a severe pyrogenic reaction with some gastrointestinal hyperactivity. Two patients suffered asthmatic dyspnoea; another developed mild congestive heart failure after the fourth injection. Albuminuria and leukocytosis were noted in all instances. Oliguria occurred only during periods of hypotension; persistent renal shutdown did not occur. Considerable tolerance to the toxic action of the material was developed in all patients.

Reactions following injection of iodinated polysaccharide did not differ appreciably from those following administration of the untreated material. The persistent post-injection exhaustion noted in many patients was greatly reduced in 2 patients by the administration of adrenal cortical extract both before and during the injection of iodinated polysaccharide.

THE EFFECT OF SIMULTANEOUS ADMINISTRATION OF BACTERIAL POLYSACCHARIDE AND ADRENAL CORTEX EXTRACT ON CELLS OF MOUSE TUMORS AND ON THE ADRENAL GLANDS OF THE HOST.

IRENE COREY DILLER. (Institute for Cancer Research, Lankenau Hospital, Philadelphia, Pa.)

In an attempt to alleviate some of the toxic effects of the tumor-necrotizing polysaccharide of Shear, Upjohn adrenal cortical extract and polysaccharide were administered simultaneously to mice (Beck and Diller, unpublished data). This did not inhibit the action of the polysaccharide on tumor tissue (sarcoma 37). The process of tumor cell degeneration, however, differed from that obtained following polysaccharide alone, and the responses occurred more slowly, reaching a maximum at about 24 hours instead of at 6. Marked fragmentation of tumor cells was apparent and unfragmented nuclei were condensed and crenulated. Acetic orcein stains these tumors very hazily and the cytoplasm is grayish and opaque, which suggests some chemical as well as structural

change. Cellular changes occur also in adrenal glands of the host, particularly in the medulla, when polysaccharide only (0.01 mgm.) or Upjohn extract only (0.5 cc. in 5 single doses) are injected intraperitoneally. Damage to the adrenal cell appears to be largely overcome when the 2 are given simultaneously.

COMPARATIVE STUDIES OF THE IMMUNOLOGICAL, TOXIC, AND TUMOR-NECROTIZING PROPERTIES OF *S. MARCESCENS* POLYSACCHARIDES. HUGH J. CREECH, MARY ALICE HAMILTON, IRENE COREY DILLER, EDWIN T. NISHIMURA, and M. J. SHEAR. (Lankenau Hospital Research Institute and the Institute for Cancer Research, Philadelphia, Pa., and the National Cancer Institute, Bethesda, Md.)

Effective clinical utilization of the tumor-necrotizing property of *S. marcescens* polysaccharide has been hindered by the concomitant toxic and immunological properties. Although these 3 properties appeared to run parallel in the earlier preparations of this polysaccharide, fractionation of one preparation yielded products in which the toxic and antigenic properties were decreased considerably, whereas the tumor-necrotizing property was not altered significantly.

Studies have been made of the effects of passive immunization of mice against the toxic action of the polysaccharide from the "G.W." strain using the γ-globulin fraction of rabbit antisera. These antibody-containing fractions upon injection into normal mice a few hours before the administration of a lethal dose of polysaccharide protected a high percentage of the animals. Injection of the γ-globulin fractions into mice bearing sarcoma 37 prior to the injection of relatively large tumor-necrotizing doses of polysaccharide afforded definite protection against the lethal action but did not seem to interfere significantly with the tumor-necrotizing action of the polysaccharide.

Two recent preparations of polysaccharide from a different strain (#724) of *S. marcescens* have been found to be less antigenic and less toxic that the preparations from the "G.W." strain; in addition, they are not related antigenically to the latter. The influence of the γ-globulin fraction of antisera toward these preparations on the toxic and tumor-necrotizing actions of the polysaccharide is being investigated.

EFFECT ON SARCOMA 37 IN TISSUE CULTURE OF TWO TUMOR-NECROTIZING AGENTS. JANE R. McCONNELL, SUZANNE F. HALLETT and M.J. SHEAR. (Institute for Cancer Research, Philadelphia, Pa., and the National Cancer Institute, Bethesda, Md.)

The action in tissue culture of a preparation of the polysaccharide from *S, marcescens* and of emetine hydrochloride was studied. Each of these agents, when injected into mice bearing sarcoma 37, produces necrosis in the tumor.

Hanging drop cultures of sarcoma 37, grown for 18 hours, were treated directly with emetine hydrochloride (concentrations of 10 mgm./cc. to 0.00001 mgm./cc). The cells became rounded; blebs formed and were pinched off; nuclei shriveled and became pycnotic. The speed of action and the degree of destruction of the cells in culture were proportional to the concentration of the emetine hydrochloride. Control experiments showed that the damage was not attributable to the hydrochloric acid. The damage seemed to be correlated with the apparent surface reducing effect of emetine on the nucleus and cytoplasm.

When hanging drop cultures of sarcoma 37 were similarly treated with the polysaccharide (concentrations of 10 mgm./cc. to 0.001 mgm./cc.), however, no necrotizing effects were observed, even though these concentrations regularly produced hemorrhage and necrosis *in vivo*.

The tissue culture method is of value in studies on the mechanism of action of such chemical agents. However, it is clear that, while it may give evidence of a direct effect of some substances on normal and malignant cells in cultures, it may not be entirely dependable if employed as a screening procedure in a chemotherapy program in lieu of *in vivo* screening.

CHEMOTHERAPY OF CANCER. CLASSES OF COMPOUNDS UNDER INVESTIGATION AND ACTIVE COMPONENTS OF PODOPHYLLIN. JONATHAN L. HARTWELL (by invitation), and M. J. SHEAR. (National Cancer Institute, Bethesda, Md.)

More than 1,200 organic compounds have been assembled for screening in tumor-bearing animals. Among the classes of compounds that have been obtained are: alkaloids; isoquinolines; derivatives of phenylethylamine, phenylpropylamine and phenylisopropylamine; carbamates; azo compounds; derivatives of phenanthrene, acenaphthene, and fluorene; diphenylethanes; stilbenes; α, β-unsaturated ketones and quinones; acridines; quaternary ammonium salts; sulfonamides; mercurials; arsenicals; and substances known to affect intermediary metabolic processes.

About 500 of these compounds have been put through a preliminary first screening in mice bearing sarcoma 37. With a few compounds, more extensive biological work has been done. The exploratory screening indicated that some classes of compounds contain a higher percentage than others of members capable of inducing damage in tumor tissue under the conditions of these experiments. For example, of 20 isoquinolines screened, only 1 gave microscopic evidence of obvious damage as compared with: 10 of 38 acridines; 23 of 211 quaternary ammonium salts; 6 of 34 arsenicals; 8 of 53 alkaloids; 4 of 53 α,-β-diphenylethylamines; 1 of 11 sulfonamides; 2 of 10 stilbenes; and 1 of 10 phenanthrene derivatives.

Podophyllin produced severe gross damage in the tumors. Fractionation yielded 2 white crystalline compounds, podophyllotoxin and a new substance designated provisionally as NCI-1074. Each of these 2 compounds possessed tumor-damaging properties in a single dose down to 3 μgm. per gm. body weight. Quercetin, another crystalline podophyllin component, yielded negative results at ten times this dose. The possible presence of

other active constituents is under investigation. Picropodophyllin, prepared from podophyllotoxin, gave negative results in doses up to 12 μgm. per gm. body weight.

NCI-1074 is isomeric with podophyllotoxin and picropodophyllin, has a melting point close to that of picropodophyllin, but is identical with neither.

HISTOLOGIC CRITERIA FOR EVALUATING THE CAPACITY OF CHEMICAL AGENTS TO PRODUCE DAMAGE RAPIDLY IN SARCOMA 37. ROSS C. MacCARDLE and VIRGINIA DOWNING. (National Cancer Institute, Bethesda, Md.)

The necrosis-producing capacity of chemical agents injected in single doses subcutaneously into mice bearing intramuscularly implanted sarcoma 37 was ascertained histologically by observing the extent and speed of changes in cells of tumor and intestinal epithelium fixed in Zenker's formol-bichromate fluid at 8, 20 and 48 hours after administration. No regression experiments were attempted in this preliminary screening of many compounds. Control tumors showed resting and dividing cells with varying amounts of spontaneous degeneration. One feature of old necrosis is the presence of extracellular bluish debris.

Tumors treated with some compounds showed extensive degeneration and necrosis in which moribund processes seemed to be in approximately the same stage, suggesting simultaneous induced injury. *Compound 368*, N-acetyliodocolchinol methyl ether, attacked tumors apparently directly, arresting mitoses in metaphase followed by necrosis. *Compound 707*, a quaternary ammonium salt, attacked tumor cells evidently directly and indirectly after vascular damage. *Compound 497*, α-phenyl-β-(3,5-diiodo-4-hydroxyphenyl)-proprionic acid, induced necrosis in some tumors. Tumors, in treated mice, showing necrosis sharply demarcated from healthy tumor tissue were considered unaffected, since control tumors occasionally presented this appearance tentatively attributed to localized spontaneous vascular blockage. *Agent 85V*, podophyllin, induced cell damage throughout the tumor apparently directly and indirectly with marked stasis and blood vessel damage. Intestinal and some tumor cells were arrested in various stages of mitosis. Mouse epidermis painted with podophyllin showed many large clear cells and polymorphic nuclei in atypical mitosis; while the intestine of the same animal showed arrested mitoses. Rous sarcoma in chickens treated with podophyllin showed induced necrosis; and cerebellar Purkinje cells were also damaged. Cell death is being studied by silver, orcein, Masson, microincineration and phase-contrast methods in these and other tumors.

THE EFFECT OF PODOPHYLLIN ON TUMOR CELLS *IN VITRO*. RICHARD A. ORMSBEE and IVOR CORNMAN. (Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

A sterile suspension of crude podophyllin, when incorporated into the nutrient medium in roller tube tissue culture preparations, exerts a toxic and repressive effect against tumor cells from the in-strain transplantable mouse tumors, sarcoma L946 A II and lung tumor MA 387. The effect on normal mouse embryonic skin growing in the same tube is negligible at concentrations which cause extensive tumor cell damage. This differential toxic effect is more marked than that obtained with any of the other known mitotic poisons which have been tested so far. This material is now being tested *in vivo* for repressive effect against a variety of tumors.

TRYPANOSOMA CRUZI IN THE TREATMENT OF MOUSE TUMORS. THEODORE S. HAUSCHKA. (Institute for Cancer Research, Lankenau Hospital, Philadelphia, Pa.)

The work of Roskin and his collaborators on destruction of tumors in animals by *Trypanosoma cruzi* "endotoxins" has recently been extended to the clinical application of Klyueva's "cancerolytic" *T. cruzi* lysates and has given impetus to related studies. Our experiments were started in March 1945 under a joint institutional program participated in by the Chemotherapy Section of the National Cancer Institute and the Laboratory of Zoology (National Institute of Health) and the Lankenau Hospital Research Institute.

Infections of *Trypanosoma cruzi* (*"B"-strain*) significantly retarded the growth of three transplantable tumors: squamous epithelial carcinoma 119, mammary adenocarcinoma, and sarcoma 37. Spontaneous breast adenocarcinoma in C3H mice was slightly retarded in growth.

The inhibitory effect was often accompanied by loss in body weight and parasitemia of vital organs. Of the 4 tumor varieties studied, only carcinoma 119 was found to be parasitized. Cancer cells proper were rarely invaded by *T. cruzi*, but parasites were relatively abundant in the stroma and in the encapsulating connective tissue. Retardation of tumor development did not result in longer survival.

Growth of carcinoma 119 was retarded or completely inhibited by infection with (1) the lethal "R-strain of *T. cruzi* (obtained from the same source as Roskin's strain); (2) a mixture of 5 avirulent strains ("A"-, "M"-, "P"-, "T"- and "C"-strain); (3) the entirely avirulent "C"-strain. Tumor-inhibition by "C"-strain was *not* accompanied by loss in body weight or other symptoms of Chagas' disease, and infected tumor-bearing mice lived longer than tumor controls.

Growth of spontaneous mammary adenocarcinoma (C3H mice) was inhibited by infection with "R"-strain *T. cruzi*. This otherwise lethal infection can be cured by treatment with the quinoline derivative, Bayer 7602.

Heat-killed cultures (50° C.) and lysates of *T. cruzi* ("B"-strain) were without effect against carcinoma 119 or mammary tumors. A lysate prepared from "R"-strain of *T. cruzi* in the plasma of infected mice contained a tumor-necrotizing "endotoxin" but also produced degenerative symptoms in liver, spleen and kidney. Test mice treated with this lysate died earlier than the controls.

THE EFFECT OF INHIBITORS OF INTERMEDIARY METABOLISM ON ADVANCED HUMAN NEOPLASIA. MAURICE M. BLACK

and ISRAEL S. KLEINER. (New York Medical College, New York, N. Y., and Brooklyn Cancer Institute, Brooklyn, N. Y.)

Malignant tissues have long been known to exhibit greater aerobic and anaerobic glycolytic activity than homologous normal tissues. Attempts to inhibit the growth of such tissues by the use of inhibitors of glycolysis by other investigators have yielded indecisive results. In view of the importance of the active phosphate bonds in energy-yielding reactions, we have attempted selective inhibition of such reactions in relation to these bonds.

The inhibitors used were sodium fluoride, iodoacetic acid, malonic acid and sodium azide. In the doses used, these substances, both singly and in combination, resulted in encouraging therapeutic effects without evidence of appreciable toxicity. The 31 cases studied, all far advanced, included acute leukemia and a diversified group of malignant tumors. Hematological and clinical remissions, for a period of 3 months, were observed in a significant number of leukemias studied. The beneficial results in patients with various types of malignant tumors included shrinkage of tumor mass, relief of pain, increase in weight and well-being, and degenerative changes in material obtained in repeated biopsies in 1 case of lymphosarcoma.

In this work, adaptation to such agents seems to be the limiting factor in continued therapeutic effect. Thus, after refractoriness to sodium fluoride and iodoacetic acid had developed, a therapeutic effect was obtained by the addition of malonic acid. Reversal of the refractory state with renewed sensitivity to the glycolytic inhibitors was also accomplished by the use of sodium azide.

The findings reported would appear to be consistent with the hypothesis of the importance of the active phosphate bond and of the possible role of accessory pathways in this phenomenon. This and other hypotheses are under investigation.

CHANGES IN THE REDUCING POWER OF PLASMA IN PATIENTS WITH MALIGNANT NEOPLASIA AND THERAPEUTIC IMPLICATIONS. MAURICE M. BLACK. (New York Medical College, New York, N. Y., and Brooklyn Cancer Institute, Brooklyn, N. Y.)

Determination of the reducing power of plasma (or serum) was made by the use of the redox dyes, brilliant cresyl blue and methylene blue. It was found that plasma of patients with malignant diseases tended to have a lowered reducing power and could usually be distinguished from normal plasma and from plasma of patients suffering from conditions other than malignancy. The decreased reducing power obtained with plasma from cancer patients tended to be grouped at different levels with individual sites of tumor origin. So far, in advanced pregnancy and in advanced hepatic cirrhosis, similar decreased reducing power has been observed. Adequate therapy (x-ray or surgery) increased the reducing power. Thus, this effect of therapy could be followed objectively.

The correlations between the results of this procedure and the diagnoses are illustrated by the following ratios. The numerator represents the number of cases showing good correlation between the clinical pathological diagnoses and the alteration in reducing power; the denominator gives the total number of cases involved. Controls (normals) 50/50; nonmalignant diseases 111/120; active malignant disease 158/184.

These observations suggested the concept that some of the symptomatology associated with malignancy might be due to the altered enzyme activity as a result of diminished -SH potential. Accordingly, glutathione or cysteine was administered intravenously. This was followed rapidly by relief of pain and general symptomatic improvement. No effect was noted, however, on the growth rate of the tumor itself.

IN VIVO STAINING OF MALIGNANT TISSUE IN MICE. MARGARET REED LEWIS and PHILIP P. GOLAND. (The Wistar Institute of Anatomy and Biology; Department of Neurosurgery, Hospital of the University of Pennsylvania; and the Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

Certain dyestuffs, namely, 3 oxazine, one thiazine, 4 xanthene, one acridine and 1 anthraquinone dye added to the diet of tumor-bearing mice stained the tumors selectively. The *in vivo* staining was accompanied by retardation of their growth.

Twenty-five dyestuffs were pulverized with fox chow in amounts equivalent to 0.4 per cent of commercial and 0.15 per cent of other compounds. Thirty mice of an inbred strain were then implanted subcutaneously with a small graft of a sarcoma native in the strain used, placed in individual containers and given 10 grams of food each day; 25 of them receiving treated and 5 untreated fox chow. Samples of urine were examined, and the mice were sacrificed about 14 days later, by which time those receiving untreated food had large tumors. As each mouse was sacrificed records were made of its condition and of the size and color of its organs and tumor. If the mouse was normal and its tumor large and uncolored the test was not repeated. If, however, its tumor was small and stained, the test was repeated. Tests on mice that became ill were repeated using less dyestuff. Compounds that stained and retarded sarcomas were tested also on mice bearing spontaneous adenocarcinomas.

Our results show that compounds that stain and retard tumors have certain structural similarities. Further investigation should disclose the structural nature of compounds less toxic and more selective in their inhibiting action on malignant tissue so that they can be synthesized for use.

STUDIES ON PURIFICATION OF THE AGENT OF CHICKEN TUMOR I. W. RAY BRYAN and VERNON T. RILEY. (National Cancer Institute, Bethesda, Md.)

Progress has been made toward purification of the agent of chicken tumor I in experiments carried out with quantitative biological assays, quantitative nitrogen determinations, and electron microscopic observations. The results of investigations involving the principles of

ultracentrifugal fractionation and chromatography were described.

RELATIONSHIP BETWEEN THE LETHAL YELLOW (A^y) GENE OF THE MOUSE AND SUSCEPTIBILITY TO SPONTANEOUS PULMONARY TUMORS. MARGARET K. DERINGER and WALTER E. HESTON. (National Cancer Institute, Bethesda, Md.)

Susceptibility to induced pulmonary tumors has been shown to be associated with the lethal yellow gene (A^y) of the mouse. Yellow F_1 hybrid mice from a cross between strains A and Y were more susceptible to pulmonary tumors induced by 20-methylcholanthrene than were their brown litter mates. In the present experiment the data indicated a relationship between the lethal yellow gene and spontaneous pulmonary tumors.

Eighty-two AYF_1 hybrids were produced and were autopsied at 15 months of age. Sixteen of the 38 yellow mice and 9 of the 44 brown mice had pulmonary tumors. The results suggested a higher degree of susceptibility in the yellow mice but were not significant. A second group of 83 mice was therefore produced and was autopsied at 15 months of age. Fifteen of the 38 yellow mice and 10 of the 45 brown mice had pulmonary tumors. These results were not significant but the combined results for the 2 groups were highly significant, $X2 = 7.425$; $P < 0.01$.

The previously demonstrated effect of the A^y gene on body size was shown by the weights of the second group at 6, 12, and 15 months of age. At 6 months the average weight for the yellow males was 14 gm. higher than that for the brown males; and that for the yellow females was 19.6 gm. higher than that for the brown females. This difference was approximately halved at 12 months and at 15 months the yellow and brown mice were of approximately equal weight.

MORPHOGENESIS AND EVOLUTION IN MALIGNANT TUMORS. SPONTANEOUS MATURATION AND REGRESSION OF TESTICULAR NEOPLASMS. NATHAN B. FRIEDMAN. (Army Institute of Pathology, Washington, D. C.)

Study of 1,000 tumors of the testis (*Mil. Surgeon*, **99**: 573-593. 1946) has revealed that teratoid growths result from the maturation of originally undifferentiated neoplasms. It is suggested that primitive cells from such tumors may metastasize before differentiation takes place, a hypothesis which would explain why teratomas lacking histologically malignant components are sometimes associated with metastases.

Some trophoblastic tumors of the testis disappear completely despite progression of their metastases. The tendency toward vascular invasion, hemorrhage and necrosis and possibly the normally brief life span of trophoblastic tissue may account for such regression. The primary site of the neoplasm remains marked by a peculiar cicatrix, which, when overlooked, leads to the erroneous diagnosis of extragenital chorioepithelioma.

Regression is not restricted to trophoblastic tumors. The tuberculoid granulomas which are common secondary stromal components of germinomas (seminomas) sometimes become more prominent than the seminomatous tissue. It is difficult or even impossible at times to identify residual neoplastic elements when the bulk of the "tumor" is made up of lymphocytes, epithelioid cells, fibroblasts and giant cells.

Teratoid tumors may be governed by oncologic principles which do not apply to other types of neoplasms. However, it might be worth investigating the new growths of other organs for evolutionary and regressive tendencies comparable to those of testicular tumors. The factors controlling neoplastic maturation and regression and the possibility of influencing them should be explored.

GENETIC FACTORS AFFECTING SYNERGISM OF LEUKEMOGENIC AGENTS. HARRY W. MIXER and ARTHUR KIRSCHBAUM. (Departments of Radiology and Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

Mice of the dba strain (subline 212) are susceptible to the induction of leukemia by either x-rays or methylcholanthrene administered independently. Although strain CBA is very susceptible to the leukemogenic action of x-rays, this stock has proved to be absolutely refractory to the induction of leukemia by methylcholanthrene.

When treatment with x-rays (1,000 r in divided doses) was combined with 18 skin paintings of methylcholanthrene dissolved in benzene (0.25 per cent solution), the incidence of induced leukemia was increased (59 per cent with combined treatment, 34 per cent with x-rays only, 33 per cent with methylcholanthrene only). When subthreshold doses of the 2 agents were combined, absolute synergism was obtained (no induced leukemia with either 6 skin paintings of methylcholanthrene, or 200 r of x-rays used alone, but 30 per cent induced leukemia when the 2 agents were combined).

Although synergism could be demonstrated in dba mice where susceptibility to each leukemogenic agent was manifest, neither synergistic nor additive effects could be obtained by combined administration of these agents to CBA mice. The incidence of leukemia was the same if 500 r were given in divided doses either alone or in combination with 18 skin paintings of methylcholanthrene.

These results suggest that leukemogenic agents may act synergistically only if the test animals are susceptible to each of these agents independently.

CHEMICAL FACTORS CONCERNED IN THE MUTUAL ADHESIVENESS OF EPITHELIAL CELLS. IRVING ZEIDMAN. (Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

Experiments were designed to determine the chemical factors responsible for maintaining mutual adhesiveness of human squamous epithelial cells. The method used depended upon separation of pairs of cells by micromanipulation, the value of adhesiveness being determined by the bend produced in a calibrated microneedle when subjected to the strain of detaching the cells. Adhesiveness was decreased in the absence of calcium or magnesium, or both. Reduction in adhesiveness brought about in a calcium-free solution was not reversed by restoring

calcium to the medium. Excess of potassium in the solution did not alter adhesiveness. Decrease in adhesiveness was produced by methylcholanthrene, a substance reported to lower the calcium content of squamous epithelium. These results offer an explanation for changes in adhesiveness recently reported in cancer cells. In these malignant cells, adhesiveness was found decreased as compared with that of normal epithelium. Since the calcium content of cancer cells has been reported to be abnormally low, it is regarded as probable that lessened adhesiveness of cancer cells is explained by their deficiency in calcium.

CHANGES OF CARBOHYDRATE METABOLISM IN PATIENTS WITH GASTRIC CANCER AND IN MICE BEARING SARCOMA 180. J. C. ABELS, C. J. KENSLER, N. F. YOUNG, and F. HOMBURGER. (Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

Studies of hepatic glycogen concentration in patients with gastric cancer and in controls undergoing laparotomies for benign abdominal disorders have shown that while the glycogen concentration is not different in the livers of patients with gastric cancer when the biopsy is taken after a 10 hour fast, it is considerably lower in the patients with cancer when both groups of patients receive glucose in the 10 hour period preceding the operation. This defect of glycogenesis can be corrected by the administration of adrenal cortical extract.

Studies on mice bearing transplanted sarcoma 180 revealed similar defects of hepatic glycogenesis. This metabolic defect is therefore independent of the type of tumor present and occurs, at least in the mouse, even when the tumor is located outside of the portal circulation.

RESPONSE OF MOUSE MYELOGENOUS LEUKEMIA TO URETHANE. ARTHUR KIRSCHBAUM and C. S. LU. (Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.)

The administration of a single anesthetic dose of urethane resulted within 24 hours in a drop in the white blood cell count and the appearance of many mature cells in the bone marrow of mice with myeloid leukemia. The depression in white blood cell count continued until 72 hours after injection, following which the counts rose. However, they had not reached the initial level 6 days after the single injection in 8 of the 11 mice tested.

The ratio of segmented to mononuclear myeloid (blast) cells in the leukemic marrow ranged from 13:87 to 46:54, with an average of 26:74. This ratio was reversed within 24 hours following a single injection of urethane (1 mgm. per gm. of body weight in aqueous solution given IP).

The number of mitotic figures in the myeloid cells of leukemic marrow was decreased following the administration of urethane. Maturation may have been secondary to inhibition of mitosis in blast cells. However, in the treated mice there were fewer marrow cells capable of undergoing division, which may account for the reduced number of division figures.

It is suggested that the release of an increased percentage of mature cells into the circulating blood may be a factor in depression of white blood cell counts following the injection of urethane into mice with myeloid leukemia.

THE METABOLISM IN THE MOUSE OF 1, 2, 5,-6-DIBENZANTHRACENE LABELED IN THE 9-POSITION With C^{14}. CHARLES HEIDELBERGER and HARDIN B. JONES. (Radiation Laboratory, University of California, Berkeley, Calif.)

Previous investigations of the metabolism of dibenzanthracene, using ultraviolet absorption spectroscopy as the analytical tool, has led to the isolation and characterization of 4′,8′-dihydroxydibenzanthracene and some preliminary information as to the distribution of this carcinogen in the animal body. This work was summarized by R. Norman Jones (*Cancer Research*, **2**:237. 1942) who made a considerable contribution to this field, and who pointed out the difficulties inherent in this method of analysis.

Dibenzanthracene labeled in the 9 position with C^{14} has been synthesized by Heidelberger, Brewer, and Dauben (*J. Am. Chem. Soc.* In press) and this material which has a specific activity 0.385 μc./mgm. is being used in an investigation of the metabolism and mechanism of carcinogenic action of this compound. Small doses of known radioactivity are administered in various ways to mice, which are kept in metabolism cages to recover the carbon dioxide of respiration as well as the urine and feces. The animals are dissected, the organs to be assayed are burned with oxygen in a combustion furnace, and the carbon dioxide is precipitated and counted in the form of barium carbonate with thin-window Geiger-Mueller counters. Dibenzanthracene has been administered to mice as a colloid in isotonic glucose, and in fat solutions.

The most striking fact observed in the metabolism of dibenzanthracene injected intravenously as an aqueous colloid, is the rapid elimination of large quantities in the feces.

A bile-fistula was performed on a mouse and after injection of the colloid, all detectable activity was present in the bile. Since there was no activity observed in the intestines or the intestinal contents, the excretion must be entirely through the bile. Chemical investigation of this bile reveals that the activity is due almost entirely to unaltered dibenzanthracene. In some cases there has been a small amount of radioactivity in the carbon dioxide of respiration, indicating the complete oxidation of at least one carbon in the molecule. This point is undergoing further investigation.

In general, it can be stated that the activity is not highly concentrated in any specific tissue of the body, but seems to be distributed fairly evenly throughout the internal organs. The mode of administration affects the amount absorbed in the body, exclusive of the gastrointestinal contents. At the end of 24 hours, 25 per cent of the activity was absorbed from intraperitoneal injection, whereas 5 per cent was absorbed from stomach-tube and from intravenous injection. When the substance is given to animals bearing highly developed mammary

carcinoma, there is no appreciable concentration in the neoplasm. When the compound is administered intraperitoneally, either in oil or as an aqueous colloid, there is a higher concentration of activity in the intestines than in the intestinal contents. This is not observed with other methods of administration, and indicates the path of absorption to be across the exposed peritoneum of the gut. Subcutaneous administration in oil indicates that at the end of six weeks, 52 per cent of the dibenzanthracene is retained near the site of injection. However, a small tumor which appeared at the site in that time interval, showed that an appreciable part of the radioactive carbon in the tumor was no longer present in the form of dibenzanthracene, but 14 per cent has been converted into an acidic form, and 21 per cent to a phenolic form. Thus 65 per cent of the dibenzanthracene is unaltered.

Aliquots of an extract of this tumor were assayed by both possible methods. The amount of dibenzanthracene calculated from the spectrographic data was twice the quantity obtained by direct radioactive assay, and this justifies previous observations that spectrographically interfering substances other than the carcinogen tend to give unreliable results.

Work is now in progress in these laboratories on the mechanism of tumor production and regression with radioactive dibenzanthracene. Distribution studies over longer periods of time, using various modes of administration, are being continued with the ultimate aim of establishing, if possible, the site and mechanism of dibenzanthracene metabolism, degradation, and elimination in the mouse.

This paper is based on work performed under Contract #W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, Calif.

CARCINOMA OF THE COLON IN RATS FOLLOWING THE FEEDING OF RADIOACTIVE YTTRIUM.

HERMAN LISCO, AUSTIN M. BRUES, MIRIAM P. FINKEL, and WALTER GRUNDHAUSER. (Metallurgical Laboratory, University of Chicago, and Argonne National Laboratory, Chicago, Ill.)

Yttrium^{91}, one of the common radioactive fission products obtained in a chain-reacting pile, is a pure beta-emitter with an energy of 1.5 mev and a half-life of 57 days. It is essentially not absorbed and, since the material remains longer in the colon than in any other portion of the intestinal tract, most of the damage occurred in this region.

One group of rats received a single feeding by stomach tube of from 1.0 to 6.0 mc. of Y^{91}. Of the 33 animals in this group, 4 died with adenocarcinoma of the colon. The earliest tumor was seen at 135 days and the latest at 506 days. Additional animals died with acute and chronic ulceration of the colon accompanied by benign and atypical hyperplasia of the mucosa.

A second group of rats was given 78 feedings of 0.46, 0.20, or 0.06 millicuries of Y^{91} per feeding over a period of 3 months. The total accumulated doses were 31.20, 15.60, and 4.68 mc., respectively. Clinically all animals appeared well during the feeding period and growth was not impaired. Six of the 8 animals at the 2 higher levels died with carcinoma of the colon from 304 to 548 days after the first feeding. No malignancies were observed at the lowest level. However, many of these animals died with superficial ulcerative lesions of the colon.

THE INFLUENCE OF COSMIC RADIATION ON THE INDUCTION OF CANCER.

FRANK H. J. FIGGE. (University of Maryland Medical School, Baltimore, Md.)

The hypothesis that the action of carcinogenic substances may be related to their efficient conversion of some form of penetrating radiation such as cosmic radiation to a form of energy capable of inducing intracellular malignant transformations was suggested by previous work. To test this hypothesis, 182 mice of inbred strains were injected with 0.25 mgm. of methylcholanthrene. They were divided into two groups and subjected to different intensities of cosmic radiation. One group consisting of 69 mice in 3 aluminum cages, used as controls, received normal, unmodified sea-level cosmic radiation. The remaining 113 mice, in 5 aluminum cages with lead plate covers, were subjected to the normal sea-level cosmic radiation plus the showers of radiation resulting from passing cosmic radiation through 1 or 2 lead plates 1 cm. thick. The average latent period for carcinogenesis in the controls was 80 days. The average latent period for induction of sarcomas in the 113 experimental mice receiving the intensified cosmic radiation was only 60 days, or three-fourths that of the controls. A repetition of this experiment gave the same results. While these results are significant, experiments to test this hypothesis in a more conclusive manner are desirable, and are contemplated.

TISSUE ELEMENTS IN THE ORIGIN OF NEOPLASMS. EVIDENCE THAT NEOPLASMS ORIGINATE AT VARIOUS LEVELS OF TISSUE ORGANIZATION.

ANDERSON NETTLESHIP. (Alexander Blain Hospital, Detroit, Mich.)

A number of early human carcinomas in various tissues were studied in order to determine the kind of immediate environment out of which cancer arises. These observations pointed to morphologic evidence that certain neoplasms have a multicellular origin. It was concluded that the majority of neoplasms originate in tissues that are in an involutionary phase and that have widespread atrophy of the parenchyma. Furthermore, such tissues may, in some areas, show hyperplasia. The degree of atrophy was usually severe. Also, a number of cases were studied that illustrate grossly and microscopically the qualitatively different type of organization manifested by neoplasms. Cases are given of (1) osteogenic sarcoma, (2) adenocarcinoma of the colon, and (3) melanocarcinoma. It is suggested that the degree of organization is dependent upon the level at which the tissue control is destroyed at the time the neoplasm is established. Another aspect, that of morphologic evidence that certain neoplasms have multicellular origin, was studied in carcinoma *in situ* of the breast, carcinoma *in situ* of the stomach, epidermoid carcinoma

of the skin and cervix uteri. In all of these it was possible to show in a focal area that the cells were approximately of the same age of neoplastic development. Additional data was submitted from tissue culture studies and induced carcinomas in lower species. In summary, it may be stated that this evidence points to the origin of cancer from tissues that have widespread atrophy. The type of cancer that is established morphologically depends upon the level at which the tissue organization breaks down.

GENETIC AND ENDOCRINE FACTORS IN ADRENAL CORTICAL TUMOR FORMATION. GEORGE W. WOOLLEY and MARGARET M. DICKIE. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

There are important genetic factors behind the occurrence of adrenal cortical tumors. This is indicated by pronounced strain differences in response to gonadectomy. The adrenal cortex of strain JAX C57 brown, for example, undergoes only slight change in size or structure following gonadectomy. Strain JAX dba develops nodular hyperplasia of the adrenal cortex and strain JAX ce, adrenal cortical carcinoma. Hybridization experiments now in progress also point to the importance of the genetic factors.

Endocrine factors are also of great influence. In our series, adrenal cortical tumors of the types described in this report did not occur without gonadectomy. Following gonadectomy certain endocrine preparations will prevent their occurrence in a genetically susceptible strain. It is known that there is an intimate relationship between the adrenal cortex and the pituitary. In these first experiments the relationship is evidenced by occasional abnormalities of the anterior lobe of the pituitary in the experimental and not in the control series.

It seems evident that there is a relationship between the adrenal cortex and the gonad, and probably between gonadotropic and adrenocorticotropic factors. An hypothesis is that with the gonad absent there is increased need of activating materials which the adrenal cortical cells may be at least partially equipped to supply. The extent to which these are needed and/or the ability of the cells to fulfill the need is undoubtedly under genetic control.

THE EFFECT OF CALORIC RESTRICTION ON THE INCIDENCE OF MAMMARY TUMORS IN CASTRATE HORMONIZED C3H MICE. CARMEN B. CASAS, JOSEPH T. KING, and M. B. VISSCHER. (Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.)

Thirty C3H mice were ovariectomized at 21 to 23 days of age. They were divided into two groups of 15 each. One group was fed *ad libitum*; the other was restricted 33 per cent in caloric intake. Both groups were fed 0.5 gamma of diethylstilbestrol daily, and both received the same absolute amounts of protein, minerals and vitamins.

Vaginal smears made by the lavage method showed a constant dense, mixed-cell picture with predominance of cornified cells in both control and experimental groups, with no recognizable difference between the two.

At the time the first tumor appeared in the control group, accidents and sacrifice of animals for tissue study had reduced the control group to 13 animals and the restricted to 10.

In the controls 3 tumors appeared in the 24th week after ovariectomy; 2 in the 27th; 1 in the 28th; 2 in the 31st and 3 in the 33rd week. Only 2 controls are tumor-free at the end of the 39th week.

Only 1 tumor has been found in the restricted animals; it appeared in the 38th week. Evidently caloric restriction did not influence the vaginal response to estrogen in these animals but did significantly alter the tumor age.

THE MILK AGENT. SAMUEL GRAFF, DAN H. MOORE, WENDELL M. STANLEY, HENRY T. RANDALL, and CUSHMAN D. HAAGENSEN. (Columbia University, and Rockefeller Institute for Medical Research, New York, N. Y.)

This communication reports our progress toward isolation and characterization of the mouse mammary carcinoma agent transmitted by and present in the milk of the high-cancer strain. Milk, a fluid of fairly constant composition, offers obvious advantages for this work. Although ordinarily a colloidal suspension of casein and fat in a solution of proteins of lower molecular weight, the milk of the high-cancer strain also contains another protein, the virus.

Fractionation by a variety of physical and chemical methods is under way. Electrophoretic, ultracentrifugal, and electron microscopic evidence on the elimination of some components, and the isolation and concentration of other components was demonstrated.

EXCRETION OF STEROIDS IN THE FECES OF MICE OF VARIOUS STRAINS WITH AND WITHOUT THE MAMMARY TUMOR MILK AGENT. LEO T. SAMUELS (by invitation), JOHN J. BITTNER, and BARBARA K. SAMUELS (by invitation). (Department of Biochemistry, University of Utah Medical School, Salt Lake City, Utah, and Division of Cancer Biology, University of Minnesota, Minneapolis, Minn.)

The excretion of ketosteroids has been studied in various strains of mice with and without the mammary tumor milk agent. It appears that the absence of the milk agent is associated with increased fecal excretion of ketosteroids. Most of the ketosteroids excreted have been found in the unconjugated form. The possible significance of the difference was discussed.

COMPARATIVE STUDIES OF THE ESTROUS CYCLES IN RELATION TO THE MAMMARY TUMOR MILK AGENT. ROBERT A. HUSEBY and JOHN J. BITTNER. (Division of Cancer Biology, University of Minnesota, Minneapolis, Minn.)

Chemical analysis of the excreta of mice has shown that mice possessing the milk agent virus have a much lower 17-ketosteroid excretion than genetically similar

mice lacking the virus (Samuels and Bittner). As androgens inhibit the action of estrogens upon the vaginal mucosa as well as upon other organs, if the altered 17-ketosteroid excretion noted is due at least in part to a change in the production and/or metabolism of androgenetically active compounds, the estrous cycles of mice should vary according to the presence or absence of the agent. To test this the estrous cycles of groups of mice differing only in this one respect were compared. Strain A and C3H mice and their F_1 hybrids and hybrids between the dba and C3H strains were studied. It was found, generally, that mice possessing the agent showed vaginal cornification a greater percentage of the time than did those mice lacking the virus. Also the percentage of vaginal cornification of mice lacking the agent could be increased by foster-nursing such mice to females that possessed the agent. Mice of the C3H strain differed from the other groups studied, for in this strain there was no difference in the estrous cycles whether the animals possessed or lacked the agent. The reason for this is obscure at the present time.

EXPERIMENTAL ALTERATION OF THE CELLS OF A TRANSPLANTED TUMOR. C. W. HOOKER, C. A. PFEIFFER, and L. C. STRONG. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

A testicular tumor composed of primitive Leydig cells that arose spontaneously in a mouse of the Strong C strain has been carried through 24 serial subcutaneous transfers in untreated mice of the same strain. No change in the cytology of the tumor has been encountered. Occasionally the grafted tumor has invaded the muscle of the body wall, and in a few instances metastases to the kidneys, liver, and lungs have been recorded. In the last 20 transfers the graft has consistently attained a size of 2.5 by 1.5 cms. in 3 weeks. When grown in castrated males the cytology of the tumor remained unchanged and the condition of the genital system of the hosts indicated no production of androgen. When equine gonadotrophin was injected daily into castrated mice carrying the tumor, the tumor cells were transformed into morphologically mature Leydig cells, and the condition of the genital system indicated the secretion of levels of androgen approximating that of the normal male mouse. Thus an agent that will provoke cellular differentiation in the normal testis has also brought about full morphological differentiation and physiological activity in the cells of an apparently malignant tumor.

EFFECT OF ORCHIECTOMY ON ADVANCED CANCER OF THE BREAST IN MALES. IRA T. NATHANSON. (From the Medical Laboratories of the Huntington Memorial Hospital of Harvard University and the Tumor Clinic at the Massachusetts General Hospital, Boston, Mass., and Pondville Hospital, Massachusetts Department of Public Health, Walpole, Mass.)

Five males with advanced, primary, recurrent or metastatic cancer of the breast were subjected to bilateral orchiectomy and exhibited some form of beneficial response therefrom. These effects were seen primarily in the original lesion, lymph node and pulmonary metastases. Changes in osseous metastases were less well defined. All the patients exhibited a general improvement in their physical status. These effects are akin to those seen in patients with advanced cancer of the prostate gland following orchiectomy or estrogen therapy. In 2 patients estrogen therapy was instituted after recrudescence of the disease. No definitive effect was seen. The response is apparently only temporary, however, as 2 patients have succumbed, and the remaining patients have shown no evidence of reactivation of their disease 9 to 15 months following orchiectomy. These findings suggests further a hormonal control of certain type of neoplasms.

METABOLIC EFFECTS OF TREATMENT OF CARCINOMA OF THE PROSTATE. JOSEPH C. AUB, DOROTHY M. TIBBETTS, and IRA T. NATHANSON. (Massachusetts General Hospital, Boston, Mass.)

Over a period of several years, we have studied metabolic effects of castration and of stilbestrol in a few patients with carcinoma of the prostate. Changes in the excretion of calcium, phosphorus, nitrogen, and citrate were not dramatic as were the variations in the blood phosphatase levels. We are trying to analyze the influence of treatment upon the viability or function of tumor cells by determining the acid phosphatase in multiple biopsies of metastatic lymph nodes.

URINARY SEX STEROID BALANCE IN PROSTATIC DISEASE. WILLIAM T. SALTER, FRANCES D. HUMM, and JOHN B. GOETSCH. (Laboratories of Pharmacology and Toxicology and the Department of Surgery, Section on Urology, Yale University School of Medicine, New Haven, Conn.)

That hormones can influence the progress of prostatic cancer is well supported by clinical and laboratory evidence. Such work has led, in general, to the theory that the affected organism presents an environment in which androgenic elements are predominant. This has led logically to methods of therapy in current practice which are aimed at upsetting the prevailing hormone balance either by (a) removal of the testis or (b) supplying exogenous estrogen, or by using a combination of both methods.

By actual test, however, the urinary excretion in such cases indicates an imbalance in the opposite direction from that which has been assumed to exist. The ratio of estrogen (in μgm.) to "androgen" (17-ketosteroids in mgm.) as determined microchemically, is strongly in favor of estrogens in a high percentage of cases of prostatic disease. The E/A ratio is under 1.0 in healthy young adult males, while ranging from 2.0 to 10 in ovulating adult females. In contrast, males with prostatic hypertrophy or prostatic carcinoma frequently show ratios in the female range, and occasionally above 10. This paradoxical trend of the steroid ratio bears no relationship to the degree of malignancy involved. It does fur-

nish evidence which indicates that the relation of androgens and estrogens to prostatic disease must be re-evaluated.

THE RELATIONSHIP OF THE NUCLEOLUS TO CYTOPLASMIC NUCLEIC ACIDS AND PROTEINS IN DIFFERENT CONDITIONS OF GROWTH IN RAT LIVER. ROBERT E. STOWELL. (Department of Pathology, Washington University School of Medicine, St. Louis, Mo., and Institute for Cell Research, Karolinska Institute, Stockholm, Sweden)

Rats were kept on a protein-free diet for periods up to 3 months to deplete the protein of the body. A few protein-depleted animals were then placed on a high protein diet for intervals up to 8 days. The liver of numerous normal controls, of 6 protein-depleted and 3 partially protein-repleted rats were frozen-dried or fixed in Stieve or Carnoy fluid. Some sections were stained with hematoxylin and eosin and others by the Feulgen reactions for thymonucleic acid. Fixed and unfixed sections were photographed with ultraviolet light of 2,570 A.

The nucleoli of the hepatic cells of rats on a protein-free diet increased to twice their normal size and the nuclei and cytoplasm decreased in volume. After a few days on a high protein diet the size of the nucleoli decreased and their number per nuclear section increased. The changes in the cytoplasmic absorption at 2,570 A were suggestive of an increased nucleotide content. The results of these preliminary experiments, when compared with similar experiments on liver cells in regeneration and in neoplastic transformation, show that there are large morphologic variations in the nucleolus of hepatic cells under different conditions of growth.

GROWTH RATE OF TRANSPLANTED TUMORS IN RELATION TO LATENT PERIOD AND HOST VASCULAR REACTION. GLENN H. ALGIRE, and FRANCES LEGALLAIS. (National Cancer Institute, Bethesda, Md.)

Transplanted tumors that have been studied in transparent chambers inserted into mice fall into two general classes in respect to their growth rate and vascular development. Among the rapidly growing group studied so far are included sarcomas, mammary gland carcinomas, and a malignant epithelial tumor of the skin. These elicited new capillary sprouts from the host as early as 2 to 3 days, the surrounding host vessels became hyperemic and numerous leukocytes accumulated about the implants. The percentage of the vascular tissue rose to approximately 50 per cent then stabilized at that level. The capillaries of the tumors mentioned above had an average diameter 5 times greater than those in a normal tissue (striated muscle), appearing as enormous sinusoid-like vessels which showed little tendency to differentiate into arterioles and venules.

In striking contrast to the rapidly growing tumors that killed the host in from 3 to 6 weeks, were the slowly growing tumors which killed the host in from 3 to 6 months. These included the Harding-Passey and Cloudman S91 pigmented melanomas, and an amelanotic melanoma derived from the S91. The slow growth rate of these tumors was correlated with a prolonged latent period prior to capillary proliferation, usually 8 days or more. In addition, vascular levels in these tumors rarely exceeded that of the vessels in the surrounding subcutaneous connective tissue, and were less than one half that of the rapidly growing tumors. There was very little leukocytic accumulation about the implants and vascular hyperemia in the surrounding tissues was lacking. The capillaries formed were small in diameter, like those of normal striated muscle, and showed considerable differentiation into arterioles and venules.

METABOLIC CHARACTERIZATION OF TRANSPLANTED MOUSE MELANOMAS BY HIGH OXIDATIVE RESPONSE TO PARAPHENYLENEDIAMINE. MARIE L. HESSELBACH, DEAN BURK, GLENN H. ALGIRE, CLARA FISCHER, and FRANCES Y. LEGALLAIS. (National Cancer Institute, Bethesda, Md.)

Tissue slices of the 3 transplantable mouse melanomas, the Harding-Passey, the Cloudman S91 pigmented melanomas and the S91A amelanotic melanoma, showed a metabolism consistent with that of malignant tumors generally, in regard to aerobic and anaerobic glycolysis, oxygen consumption, respiratory quotient, and other related derived quotients.

On the other hand, all 3 melanomas showed a much greater percentage stimulation (400 to 1,000) of oxygen consumption by paraphenylenediamine than any other tumors tested to date (0 to 150 per cent), and the stimulation was in all cases essentially eliminated by cyanide. This greatly enhanced stimulation of oxygen consumption by paraphenylenediamine offers the possibility of a biochemically new characterization and mode of diagnosis of melanomas, amelanotic as well as pigmented, that is readily subject to further testing with a variety of other melanomas.

The marked stimulation of oxygen consumption caused by paraphenylenediamine in cyanide-free tissue slices of the melanomas, as compared with other tumors tested, may be interpreted as indicating that the ratio oxidized/reduced cytochrome *c* is considerably higher in these melanomas than in other tumors, and, in fact, in the range in normal and embryonic tissues generally. This is indicative of a relatively high level of oxidation-reduction potential within the melanoma cells, either intracellularly throughout or locally in certain cell areas.

THE EFFECT OF AGE ON REGENERATION OF RAT LIVER FOLLOWING PARTIAL HEPATECTOMY. NANCY L. R. BUCHER, ANDRÉ GLINOS, and JOSEPH C. AUB. (Medical Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital, Boston, Mass.)

It seems possible that the frequent association between cancer and old age may express some significant aspect of the genesis of cancer. Accordingly it seems important to formulate the response of ageing tissues to growth stimuli.

Regenerating rat liver has been chosen for such a study because its restorative capacity can be accurately quantitated. Previous investigators have found that age delays mitosis in this tissue, and that it retards restoration of liver.

In the present experiment rats of accurately known ages were divided into young (4 to 6 weeks), adult (6 to 8 months) and old (1¾ to 2½ years) animals. Many of the latter group showed the characteristics of senility. The main lobes of the liver, constituting approximately 68.4% of the total, were removed, and the method of Brues, Drury and Brues (*Arch. Path.*, **22**:658. 1936) followed in determining the percentage of restoration in terms of (1) mass and (2) number of cells. Rats were autopsied at intervals of 16 and 30 hours, and 3, 7 and 14 days after operation.

The young rats, in whom regeneration was superimposed on active growth, were not strictly comparable to the other two groups. In general the regenerative capacity decreased with age. Restoration of liver mass was retarded in the adult and old rats as compared with the young rats, and restoration of hepatic cells to their original number was retarded in the old rats as compared with the other two groups.

THE CITRIC ACID CONTENT OF TUMOR TISSUE AND OF TUMOR-BEARING ANIMALS.

FRANCES L. HAVEN, and CHALLISS RANDALL. (Department of Biochemistry, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

The citric acid content of Walker 256 and of liver, blood, and kidneys of rats bearing this tumor has been determined. The necrotic portion of 21 tumors contained 4 to 20 times more citric acid than the non-necrotic portion. The blood of tumor-bearing animals was normal in citric acid. The kidneys and, to a lesser extent, the livers of rats bearing this tumor were higher in citric acid than similar organs of rats without tumors.

ON DEFECTIVE PLASMA PROTEIN FORMATION IN PATIENTS WITH GASTRIC CANCER.

F. HOMBURGER, AURELIA POTOR, and N. F. YOUNG. (Sloan-Kettering Institute for Cancer Research, New York, N. Y.)

Intractable hypoproteinemia is part of the systemic disease found in patients with gastric cancer. This study of nitrogen balance and plasma protein regeneration was made on 13 patients with gastric ulcers, operable and inoperable cancer of the stomach. It was found that on high protein intakes, sufficient to produce plasma protein regeneration in patients with gastrectomies for ulcers, plasma protein levels of patients with gastric cancer remained low or continued to fall. The increase of circulating plasma protein in 2 cases was due to globulins. This finding, together with the fact that even in the presence of positive nitrogen balance for as long as 80 days no increase of circulating plasma protein occurred, suggests an anomaly of protein metabolism in these patients.

HISTOCHEMICAL PHOSPHATASE REACTION IN MOUSE SARCOMAS CR 180 AND 37 FOLLOWING ADMINISTRATION OF BACTERIAL POLYSACCHARIDE.

MORRIS BELKIN, and ELMER D. BUEKER (by invitation). (Departments of Pharmacology and of Anatomy, Medical College of South Carolina, Charleston, S. C.)

Swiss albino mice carrying 2 weeks old implants of sarcoma CR 180, and dba mice carrying similar implants of sarcoma 37 were each given 0.1 mgm. of bacterial polysaccharide intraperitoneally. They were then sacrificed, as were control animals, in groups of 2 or 3, at half-hourly or hourly intervals for the first 4 hours, and at 8, 12 and 24 hours after injection. The tumor tissue was fixed in chilled acetone for acid phosphatase, and in 80 per cent alcohol for alkaline phosphatase preparations. Gomori's histochemical method was used for both acid and alkaline phosphatase reaction, with minor modifications.

For both acid and alkaline phosphatases, 5 different substrates were used, with varying incubation periods as follows:

	Acid pH 5, hours	Alkaline pH 9.4, hours
Glycerophosphate	6½	5
Adenylic acid	72	3
Fructose diphosphate	72	2
Lecithin	72	72
Yeast nucleic acid	48	3

No striking effects were obtained, for any of the substrates, for either acid or alkaline phosphatase reaction.

The tumors incubated with glycerophosphate and yeast nucleic acid on the acid side, and all the substrates on the alkaline side, showed a mild increase in staining properties 2 to 3 hours after polysaccharide administration.

Microscopically, at this interval, the nuclear wall and nucleoli were somewhat more darkly stained. But, as the cytotoxic action of the polysaccharide continued, with progressive dissolution of nuclear contents into granular fragments, and their dispersal into the cytoplasm, the intensity of the staining diminished.

It is concluded that the cytotoxic effect of bacterial polysaccharide is not mediated through attenuation or destruction of the phosphatase enzymes in so far as it has been studied by this particular histochemical technic.

REDUCTION IN TOXICITY OF *SERRATIA MARCESCENS* POLYSACCHARIDE TO TUMOR-BEARING MICE PRODUCED BY UPJOHN CO. BEEF ADRENAL EXTRACT.

LYLE BECK, IRENE DILLER, BERTINA BLAUCH, and MARY FISHER. (Lankenau Research Institute, Philadelphia, Pa.)

The *Serratia marcescens* tumor-necrotizing polysaccharide isolated by Shear and his co-workers (*J. Nat. Cancer Inst.*, **4**) produces toxic effects which may culminate in death when the mice bear large tumors or when a dose many times that required to produce extensive necrosis is given to mice bearing small tumors.

Five hundred micrograms of *S. marcescens* polysaccharide preparation P_s of the National Cancer Institute

caused death within 48 hours in 64 of 75 mice bearing 7 day tumors (sarcoma 37). Most of these mice died within a few hours after intraperitoneal injection of the polysaccharide. Another group of 45 mice bearing 7 day tumors was given 0.25 cc. of Upjohn Co. beef adrenal extract, 500 μgm. of P_s polysaccharide and another 0.25 cc. of adrenal extract at the end of the working day. Of these mice, 22 survived 48 hours or longer. The probability of this difference in survival being due to chance was calculated using Chi Square and was found to be about 1 in 10,000.

On the other hand, no evidence was secured that Upjohn Co. concentrated hog adrenal extract in oil is effective in counteracting the lethal effects of 500 μgm. of polysaccharide preparation P_s given to mice bearing 7 day tumors.

STUDIES OF PULMONARY TUMOR INDUCTION IN MICE BY DERIVATIVES OF CARBAMIC ACID. C. D. LARSEN. (National Cancer Institute, Bethesda, Md.)

Examination of the phenomenon of pulmonary tumor induction in mice by ethyl carbamate and other derivatives of carbamic acid have been extended. In strain A mice single injections of a narcotizing dose of ethyl carbamate initiated increases in incidence and frequency of lung tumors. Although an initial response was noted after 1 month, maximum effects were not observed until 5 months had elapsed.

Ethyl carbamate (urethane) was relatively specific in its capacity to induce lung tumors. Studies of ester homologues of urethane, other than those previously reported, substantiate the specificity of urethane. β-chloroethyl carbamate and trichloroethyl carbamate were inactive; propyl carbamate and isopropyl carbamate exhibited about 1 and 5 per cent, respectively, of the activity of the ethyl ester. *N*-alkylated ethyl carbamates, with one exception, tended to decline in activity as the extent of alkylation was increased. Mono-*N*- and di-*N*-methyl ethyl carbamates exhibited only about 10 and 5 per cent, respectively, of the activity of urethane. Mono-*N*-isopropyl ethyl carbamate, however, elicited a striking increase in lung tumors; an activity approaching 50 per cent of that of urethane was noted.

Embryonic lung tissue was found susceptible to the oncogenic action of urethane. Litters from pregnant mice that had been injected with a single narcotizing dose of urethane prior to parturition were kept until 6 months of age. Striking increases in the incidence and multiplicity of lung tumors in the offspring were observed. Essentially identical results followed either intraperitoneal or intravenous injection of the pregnant mice. The response of pulmonary tissue to *in utero* exposure to the agent tended to vary inversely with the injection-parturition interval.

AN EXPERIMENTAL STUDY OF SINGLE TRAUMA MALIGNANCY. WILLIAM L. SIMPSON. (The Barnard Free Skin and Cancer Hospital, and Washington University School of Medicine, St. Louis, Mo.)

The skin of the Swiss strain mouse is rendered unusually susceptible to the action of carcinogenic agents by the prolonged application to it of carcinogenically inactive solutions of methylcholanthrene in anhydrous lanolin. Unless actively carcinogenic compounds are applied to the skins of these "sensitized" mice, they usually remain free from cancer until death, and neither structurally nor chemically do their skins resemble those in which precancerous changes have been initiated by "active" solutions of methylcholanthrene in benzene.

Experiments have been conducted on such hypersusceptible skin, as well as on normal mouse skin, to test the cancer-evoking potentialities of a single severe trauma. Three types of injury were inflicted: (1) burning with a hot glass rod, (2) crushing of the skin with pliers, and (3) exposure to a massive localized dose of roentgen irradiation.

Only rarely does the normal mouse respond to a solitary trauma by the development of a malignant tumor, a result in agreement with most earlier observations. In sharp contrast are the results on "sensitized" mice. Malignant tumors did not follow injuries by crushing or by x-ray "burns," but in two groups of mice subjected to trauma by burning with a hot glass rod malignant tumors appeared in 42 per cent and 65 per cent respectively. Carcinomas, sarcomas and carcinosarcomas were produced. In both groups approximately 80 per cent of the tumors arose *at the site of the preceding injury.* The average period of induction, dated from the time of injury, was 7 months.

The bearing of these experiments on the much discussed problem of single trauma cancer in man was considered.

DIFFUSIBLE AND NON-DIFFUSIBLE CALCIUM IN NORMAL AND METHYLCHOLANTHRENE-TREATED MOUSE EPIDERMIS. A. I. LANSING and M. H. AU. (Department of Anatomy, Washington University School of Medicine, and Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

Carruthers and Suntzeff in 1943 established that epidermal calcium is significantly decreased in methylcholanthrene-induced hyperplasia and carcinoma. The present investigation was designed to explore further this pronounced shift in total calcium and to determine whether the ratio between free and bound calcium in early and late hyperplasia and carcinoma is altered.

The method employed for separation of free and bound calcium (measured as diffusible and non-diffusible calcium) was based upon the ultrafiltration technic of Mazia in 1937, and calcium was determined by the method of Lindner and Kirk, the same year.

The ratio of diffusible to non-diffusible calcium in normal 3 month old Swiss mice was 1:1.6; early (20 days) hyperplasic epidermis revealed no significant alteration of this ratio but confirmed the 50 per cent drop in total calcium reported by Carruthers and Suntzeff. Study is being made of the diffusible and non-diffusible calcium ratio in late hyperplasia (60 days) and carcinoma.

HYALURONIDASE AND THE GROWTH OF MALIGNANT EPITHELIAL TUMORS. A. R. GOPAL-AYENGAR, and WILLIAM L. SIMPSON. (The Barnard Free Skin and Cancer Hospital, and Washington University School of Medicine, St. Louis, Mo.)

Although an association between "spreading factors" and the growth of malignant tumors has been recognized for some years, the nature of the relationship has never been elucidated. We have now tested by direct experiments the hypotheses formulated in 1943 by Cramer and Simpson on a possible mechanism for this association. Included in the investigations were: (1) the effects of a spreading factor from testis (hyaluronidase) on the growth and invasive capacities of a mouse-transplantable squamous cell carcinoma, (2) the relation of hyaluronidase and anti-hyaluronidase antibodies to the development of the transplantable tumor, and (3) the effect of the enzyme on carcinogenesis in response to methylcholanthrene.

Local injection of the hyaluronidase about the base of established cancer transplants resulted in the enhancement of invasive growth with a striking destruction of muscle and bone by the tumor. In a few instances the local injection was followed promptly by the appearance of distant metastatic lesions.

Results of the other experiments, which were not then quite completed, were described at the meeting.

THE ROLE OF SEBACEOUS GLANDS AND HAIR FOLLICLES IN EPIDERMAL CARCINOGENESIS IN MICE. V. SUNTZEFF, C. CARRUTHERS, and E. V. COWDRY. (From the Barnard Free Skin and Cancer Hospital, and the Department of Anatomy, Washington University School of Medicine, St. Louis, Mo.)

Previous studies in this laboratory revealed that young New Buffalo mice developed squamous cell carcinoma more rapidly and in a higher percentage than did old mice of the same strain after the topical application of methylcholanthrene. This difference led to an investigation of the response of the skin of very young mice (2 to 10 hours after birth) to a single application of the same carcinogen. Thirty mice were treated in this fashion, and 19 months after the application of the carcinogen, 23 mice were alive without evidence of tumor formation. A possible morphological basis for this lack of reponsiveness was found in a detailed study of the development of the skin and its associated structures from the time of birth until the skin was completely developed. The hair follicles and sebaceous glands were found to be rudimentary at the time the carcinogen was applied, and the epidermis was well differentiated and covered with a thick layer of keratin. The failure of very young mice to develop cancer may be due to the following factors: Inability of the carcinogen to penetrate through the thick epidermis or to reach the few rudimentary sebaceous glands via the hair follicles, only a few of which have hair reaching the exterior. That the hair follicles and sebaceous glands play an important role in epidermal carcinogenesis in mice is quite apparent from this study.

STUDIES ON THE TRANSMISSION OF AVIAN VISCERAL LYMPHOMATOSIS. I. VARIATION IN TRANSMISSIBILITY OF NATURALLY OCCURRING CASES. BURMESTER, B. R., and DENINGTON, E. S. (U. S. Regional Poultry Research Laboratory, East Lansing, Mich.)

The transmissibility of tumors from 10 cases of naturally occurring visceral lymphomatosis was tested by inoculation of cellular and cell-free preparations into groups of 14 to 21 chicks 1 day of age. The recipient chicks were relatively free from prior infection since none of 41 noninoculated controls developed tumors during an experimental period of 183 days.

Lymphomatous tumors of the viscera were reproduced (an incidence of 14 to 85 per cent in 93 to 183 days) in recipients of cell-containing preparations from 8 of the original tumors. Similar tumors were produced (an incidence of 39 to 94 per cent in 183 days) by cell-free preparations from 5 of the original tumors. In addition to the visceral tumors, preparations from 1 tumor also produced a high incidence of osteopetrosis.

Of the 10 donors that supplied visceral tumors, 7 also had gross or microscopic evidence of neurolymphomatosis. Gross neural lesions appeared in 1 to 4 chickens of several groups; however, there appeared to be no direct relation between the presence of this lesion in the donor and the number of recipients that developed neural or visceral lymphomatosis.

Tumors of some, but not all, cases of visceral lymphomatosis are transplantable, and part of these tumors may be transmitted to chicks by inoculation with filtrates. The active agent or agents are of a size which will allow them to pass readily through bacteria-retaining filters.

TRANSPLANTATION OF THE ROUS CHICKEN SARCOMA INTO THE ANTERIOR CHAMBER OF THE MOUSE EYE. EDWARD W. SHRIGLEY. (From the Department of Bacteriology and Immunology, Yale University School of Medicine, New Haven, Connecticut)

The Rous chicken sarcoma placed into the eye of the mouse grows to fill the chamber and frequently herniates to the exterior through the cornea. The growth behavior of the sarcoma in the mouse eye is similar to that in the eye of the guinea pig. However, in the former the tissue persists longer before undergoing regression. Transplants capable of producing growths in chicks have not been obtained from mice after 15 days of residence. Chicks injected directly with this mouse growth may show, in addition to the local tumor, hemorrhagic disease and periosteal sarcomas. Subsequent passages in chicks indicate that unlike the guinea pig passage agent, the virus has not undergone alteration in specificities nor has it increased in potency. On the contrary, data suggest that the mouse passage virus has lost some of its virulence while its tissue specificities are no different from those of the stock Rous agent.

THE MORPHOLOGIC STABILITY OF SIX STRAINS OF MALIGNANT MOUSE FIBRO-

BLASTS GROWING *IN VITRO*. WILTON R. EARLE. (National Cancer Institute, Bethesda, Md.)

The production of six strains of sarcoma cells from one parent strain of mouse fibroblast growing in an entirely heterologous medium *in vitro* has been previously reported. Of these six, strains H, J, L, N and O had been treated with a concentration of 1 μgm. of 20-methylcholanthrene per ml. of culture media for 6, 32, 111, 184, and 406 days respectively. The degree of morphologic alteration in these cell strains was apparently directly associated with the time the cell strains had been subjected to the carcinogen. Strain D, the presumably untreated control strain, also underwent a very limited morphologic alteration, but never showed as great a change as the cells of strain H, which were subjected to the carcinogen for 6 days.

The last of these cell strains was removed from 20-methylcholanthrene on September 16, 1942, and since that time all strains have been grown in the same heterologous culture medium of chicken plasma, horse serum, and chick embryo extract, and under the same experimental culture conditions.

Periodic photographs of these living cultures from December 17, 1942, through December 19, 1946, showed that strains J, N, and O have undergone certain limited secondary alterations within this interval. Strains D, H, and L, however, have shown no recognizable change in their respective characteristic induced morphologies since December 17, 1942. Allowing generously 5 days for each intermitotic interval in these three cell strains, it seems that the respective characteristic induced morphologies of these three cell strains have been stable for over 290 consecutive cell generations.

THE USE OF PURIFIED FIBRINOGEN WITH CERTAIN STRAINS OF NORMAL AND MALIGNANT FIBROBLASTS IN TISSUE CULTURES. VIRGINIA J. EVANS, HELEN M. DYER, and MARGARET G. KELLY. (National Cancer Institute, Bethesda, Md.)

An attempt was made to obtain a more chemically reproducible solid culture medium for tissue culture metabolic studies than has been possible by the use of plasma. A study has been made of bovine fibrinogen prepared by a number of different procedures. Test cell strains used have all been subcutaneous mouse fibroblasts and have included 3 strains of presumably normal mouse fibroblasts, one freshly explanted *in vitro* and two grown *in vitro* for more than 3 years. Earle's sarcoma strains D, H, J, L, N, and O were also used. All cultures were grown in Carrel D3.5 flasks and the supernatant culture medium has been 40 per cent saline, 40 per cent horse serum and 20 per cent chick embryo extract.

Results to date indicate that different cell strains vary substantially in their tendency to lyse this solid substrate. Of the cell strains tried, strain L, alone was unable to lyse the clot to any perceptible degree. All three strains of normal cells showed rapid lysis of the clot as did the sarcoma strains D, H, J, N, and O.

HEREDITARY EOSINOPHILE LEVELS IN THE ACQUIRED RESISTANCE OF THE RABBIT TO THE BROWN-PEARCE TUMOR. ALBERT E. CASEY and GEORGE R. DRYSDALE. (Department of Pathology, The Baptist Hospital, Birmingham, and the Holy Name of Jesus and Baptist Memorial Hospitals, Gadsden, Ala.)

Previous studies by our group demonstrated hereditary variations in the blood eosinophile levels of normal rabbits but none for the neutrophiles or monocytes. High pretransplantation eosinophile levels were associated with a lower incidence and number of metastases, and a lower mortality in animals receiving successful transplants than low pretransplantation levels. No such relation for the neutrophile or monocyte levels could be demonstrated.

Because the eosinophile effect did not seem to become manifest until the seventh week after inoculation 98 additional animals were studied, giving a cumulative total of 283 young adult male rabbits received from breeders. Of these, 159 had the blood level of each of nine blood cell factors within normal limits for the species, and were seemingly free from intercurrent disease. These 159 normal animals were inoculated intratesticularly with the Brown-Pearce tumor, and surviving animals were sacrificed two months thereafter.

The cumulative data indicate that the pretransplantation eosinophile level bears no apparent relationship to the course of the Brown-Pearce tumor during the first six weeks after inoculation. Its effect appears in the seventh week and persists with the characteristic and statistically significant pattern described above. It especially seems to affect the incidence of hematogenous metastases.

The seventh week corresponds to the beginning of regression or the turning point of this neoplastic disease as first described by Brown and Pearce and later by Malluche. Thus a relationship between the hereditary eosinophile level and the acquired resistance of the rabbit to the tumor is indicated.

RETARDATION OF GROWTH AND METABOLISM OF NORMAL AND MALIGNANT CELLS DURING CONTINUOUS CULTURE. JOHN H. HANKS (by invitation), GEORGE O. GEY, and RACHEL BARRETT (by invitation). (Division of Cell Physiology, Department of Surgery, Johns Hopkins Hospital and Medical School, Baltimore, Md., and Leonard Wood Memorial Department of Bacteriology, Harvard Medical School, Boston, Mass.)

Throughout the life of multicellular organisms, most of the tissues and organs are capable of carrying on their specific function with a fairly stable cell population and, therefore, at a low maintenance rate of growth. When cells are released from the organization and control of the host and are explanted in tissue cultures, conditions are usually provided which cause them to migrate and divide rapidly. Since a major portion of biological and medical interest in the results of tissue cultivation depend

on interpretation in terms of post-embryonic or adult physiology and pathology, it is obvious that the art and science of tissue cultivation need some reorientation in the direction of maintaining more stable populations of cells without rapid multiplication. Maintaining cells at metabolic levels approximating those of postpartum physiology is of value in studying problems concerned with cytology, nutrition, endocrine secretion, antibody formation, the interrelations of cells and infectious agents, and the riddle of differentiation and malignancy. By lowering cell metabolism through reduction in temperatures of maintenance and by decrease in concentration of nutrients, it has been possible to perpetuate strains of normal and malignant cells over long periods of time with minimal effort. Reduced temperature levels thus far investigated include 28°, 31°, and 34° C. The results reported include studies on normal human and rat fibroblasts and several strains of rat sarcoma. The effects of lowering temperature and nutrient supply upon rate of growth, duration of mitosis, cultural behavior, and cytology were discussed.

FURTHER OBSERVATIONS ON THE CONVERSION OF NORMAL INTO MALIGNANT CELLS *IN VITRO*. GEORGE O. GEY, and MARGARET K. GEY (by invitation). (Division of Cell Physiology, Department of Surgery, Johns Hopkins Hospital and Medical School, Baltimore, Md.)

This study is concerned with a series of permanent alterations occurring in continuous cultures of normal rat mesenchyme cells and leading to the production of malignant cells. The strains studied include normal, altered normal, and malignant cell strains of *autologous* origin which have been under cultivation for eight and one-half years. It has been possible to make direct comparison between a normal and a malignant strain derived from it. The data to the present time implicate factors contributed by a culture medium totally heterologous to the strains studied. No known extraneous carcinogenic agents have been found to play a part in these conversions which occurred in stocks of normal cell strains. Differences between normal and malignant autologous strains were discussed.

IS AEROBIC GLYCOLYSIS OF AN INTENSITY CHARACTERISTIC OF CANCER TISSUE A NORMAL METABOLIC FEATURE OF THE MUCOSA OF THE SMALL INTESTINE? OTTO ROSENTHAL. (Harrison Department of Surgical Research, School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

In 1941 Dickens and Weil-Malherbe reported that the rate of aerobic glycolysis of normal duodenal or jejunal mucosa of rat and mouse equals that generally obtained with cancer tissue. These authors believed they had eliminated the possibility of an artefact in spite of the exceptional observation that the aerobic glycolysis was as high as the anaerobic glycolysis. Such complete absence of the Pasteur effect has never before been observed in undamaged tissues whether normal or malignant.

Since the mucous membranes of murine species are extremely fragile the metabolism of the more stable duodenal mucosa of the rabbit was studied manometrically by means of Warburg's indirect method. In addition, lactic acid was determined colorimetrically with the method of Barker and Summerson.

The rates of respiration and of anaerobic glycolysis of the rabbit mucosa approximated those obtained with duodenal mucosa of the rat by Dickens and Weil-Malherbe. Q_{O_2} and $Q_G{}^{N_2}$ averaged 9.5 and 7.9 respectively (initial dry weight basis, 60 minutes). The aerobic glycolysis, however, amounted to but 10 per cent of the anaerobic glycolysis. The Pasteur effect was thus evident. Persistence of a small aerobic glycolysis is commonly found with normal tissues *in vitro*.

While these results do not eliminate the possibility that the high aerobic glycolysis of murine mucosa of the small intestine is a peculiarity of the species, the known absence of species differences in the metabolism of colonic mucosa does not favor this interpretation, but rather suggests an artefact.

MICROMETRIC INVESTIGATIONS ON MYELOMA CELLS AND NORMAL BONE MARROW PLASMA CELLS. HARALD GORMSEN. (Department of Pathology, University Institute of Forensic Medicine, Copenhagen, Denmark)

Micrometric investigations by ocular micrometer have been carried out on normal bone marrow plasma cells (smears of sternal punctures and sections of bone marrow from 15 normal adult persons) and on myeloma cells (smears of sternal punctures and sections of myeloma tissue from 29 patients). In each preparation 50 cells and their nuclei have been measured in longitudinal and transverse direction. The average values of the 50 measurements have been subjected to statistical analysis.

In 18 of the 29 cases of myeloma both the nuclei and total cell size were significantly larger than normal plasma cells in bone marrow. In 8 cases, only the nuclei of the myeloma cells were significantly larger than nuclei of normal plasma cells in the bone marrow, whereas 3 myeloma cases showed cell- and nuclei-sizes that did not differ from normal bone marrow plasma cells.

Consequently, in the majority of myeloma cases (in the present material 26 out of 29) the myeloma cells differ unmistakably from normal bone marrow cells. In a few cases (in the present material 3 out of 29) myeloma cells in all aspects (cell size, nucleus size, nuclear structure, etc.) are morphologically identical with normal bone marrow plasma cells.

This observation is of practical importance in the use of sternal punctures for the differential diagnosis between myelomatosis and conditions with reactive plasma cell proliferation in the bone marrow (infections, etc.).

No significant relation could be demonstrated between the degree of morphological abnormality of myeloma cells and the clinical symptoms or the course of the myeloma cases.

THE EFFECT OF SOME CARCINOGENIC AMINOAZO DYES ON THE AUTOXIDATION OF LINOLEIC ACID. H. P. RUSCH, and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Previous publications from this laboratory have demonstrated that carcinogens including certain azo dyes inhibit the autoxidation of unsaturated lipids. The present paper describes in more detail the effect of *p*-dimethylaminoazobenzene and its demethylated derivatives on the autoxidation of purified linoleic acid. The aminoazo dyes were purified by chromatographic adsorption. Known quantities of linoleic acid and the azo dyes were placed in Warburg flasks and the rate of autoxidation was followed manometrically at 36.5° C. The flasks contained linoleic acid alone or with varying levels of *p*-dimethylaminoazobenzene (DAB), *p*-monomethylaminoazobenzene (MAB), or *p*-aminoazobenzene (AB).

DAB and MAB both increased the latent period of oxidation of linoleic acid, the former being a more effective antioxidant than the latter, and the antioxidant effect of each dye was proportional to the concentration employed. Thus, when DAB was used at concentrations of M/200, M/100, and M/50 the oxidation of the linoleic acid at the end of the first 24 hour period had progressed only 56, 29, and 0 per cent respectively as compared to the acid alone. With the same levels of MAB, the amount of oxidation was 73, 45, and 35 per cent respectively. Contrary to the inhibiting effect of the methylated dyes, AB shortened the latent period slightly.

As the autoxidation proceeded, the azo dyes disappeared from the flasks and DAB and MAB were found to be demethylated. At the end of 30 hours 90 per cent of the DAB initially added had disappeared from the flask but MAB appeared in amounts equal to 85 per cent of the starting level of DAB. Small amounts of AB were also found throughout the run. MAB disappeared more slowly than DAB during the course of the oxidation and it was found to be demethylated to AB. AB disappeared very rapidly in oxidizing linoleic acid and no other basic dye was detected in the mixture.

THE INHIBITION OF THE GROWTH OF *LACTOBACILLUS CASEI* BY *P*-MONOMETHYLAMINOAZOBENZENE AND ITS REVERSAL BY RIBOFLAVIN. E. C. MILLER, H. N. KINGSLEY, and J. A. MILLER. (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

The activity of the hepatic carcinogens *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene can be greatly modified by the character of the diet in which they are fed. In particular, feeding diets high in riboflavin to rats greatly delays tumor development due to these compounds. This antagonism has now been studied using the growth of *L. casei* as the end point; the growth of this organism is proportional to the riboflavin content of the medium. The bacteria were grown in the medium of Roberts and Snell except that in certain cases amino acids were substituted for a part of the hydrolyzed casein. Growth was measured turbidimetrically 24 hours after inoculation. *p*-Monomethylaminoazobenzene was generally used because it is 10 times as soluble as *p*-dimethylaminoazobenzene in aqueous media.

When 1 to 3 μgm. of *p*-monomethylaminoazobenzene were added per ml. of medium, growth was inhibited by 60 to 90 per cent at riboflavin levels of 0.01 to 0.15 μgm. per ml. Increasing the level of riboflavin to 1.25 μgm. per ml. decreased the inhibition to 0 to 40 per cent; higher levels of riboflavin were impractical because of the poor solubility of the vitamin. The inhibition due to the dye could also be reversed by an unidentified constituent present in fresh pancreatic digests of casein; the activity of this factor decreased on storage in the cold for 4 to 8 weeks. When *Saccharomyces cerevisiae* was grown anaerobically in the same medium, its growth was inhibited 20 to 40 per cent at levels up to 0.2 μgm. of riboflavin per ml. Larger amounts of riboflavin usually reduced the inhibition to 10 per cent or less. *S. cerevisiae* destroyed 80 to 90 per cent of the *p*-monomethylaminoazobenzene in the medium at the high levels of riboflavin while *L. casei* destroyed only 10 to 20 per cent.

SUSCEPTIBILITY OF STRAIN C MICE TO *o*-AMINOAZOTOLUENE. H. B. ANDERVONT, and THELMA B. DUNN. (National Cancer Institute, Bethesda, Md.)

Female mice of strain C are much more susceptible than males to hepatic lesions induced by *o*-aminoazotoluene. Castration of males considerably increases their susceptibility while castration of females lowers their susceptibility. Administration of testosterone propionate to castrated males or females lowers their susceptibility to that of intact males. The compound induces hemangioendotheliomas in both sexes. The site of origin of these tumors is influenced by the site of administration of the compound.

TUMORS PRODUCED IN RATS AFTER INGESTION OR PAINTING OF 2-NITRO, 2-AMINO, *N*-ACETYL-2-AMINO, AND *N*-DIACETYL-2-AMINO FLUORENE. H. P. MORRIS, C. S. DUBNIK, T. B. DUNN, and J. M. JOHNSON. (National Cancer Institute, Bethesda, Md.)

The carcinogenic effect on the rat of 4 derivatives of fluorene were studied after both ingestion and painting. In the feeding experiments each derivative was fed to rats at a level of 0.05 per cent in a low-fat synthetic diet for 160 days. The average daily ingestion of carcinogen ranged from 4.0 to 4.7 mgm. In the painting experiments a 2 per cent acetone solution of each compound was applied thrice weekly to the scapular region. The estimated amount of carcinogen given with each application was 0.5 mgm. during the first 6 months and 1.00 mgm. thereafter. The painted rats were fed a stock diet. Autopsies were made after the appearance of tumors or

when death appeared imminent. Sixty-four tumors were identified histologically in 104 treated animals. No tumors were found in control animals.

Distant tumors were produced by all 4 derivatives after either ingestion or painting. 2-Aminofluorene was the only compound producing skin tumors. The majority of liver tumors were observed in rats after ingesting either the mono or diacetyl derivative. The 2-nitro derivative induced no liver tumors. The results of these experiments suggest for both types of administration an increasing order of carcinogenicity from the nitro to the amino to the mono or diacetyl derivative. The type of distant tumors produced, while not dependent on the route of administration, seems to be influenced by it.

PARALLEL EFFECTS OF CERTAIN DIETS UPON THE RETENTION OF RIBOFLAVIN AND THE FORMATION OF HEPATIC TUMORS IN THE LIVERS OF RATS. A. C. GRIFFIN, and C. A. BAUMANN. (Department of Biochemistry, University of Wisconsin, Madison, Wis.)

When *p*-dimethylaminoazobenzene was fed to rats, the amount of riboflavin in the liver varied with the concentration of this vitamin in the diet: liver storage was lower on synthetic or semi-synthetic diets containing 0.7γ of riboflavin per gm. of diet than on similar diets containing 2.0γ of riboflavin per gm. The rate of tumor formation was faster on the lower level of riboflavin intake, and on any one level it was essentially the same whether the other B vitamins were supplied as a synthetic mixture or as a crude rice concentrate. In the presence of *m'*-methyl-*p*-dimethylaminoazobenzene the hepatic storage of riboflavin was low on both dietary levels of the vitamin; and previous studies have indicated that tumors due to *m'*-methyl-*p*-dimethylaminoazobenzene form at essentially the same rate on either diet.

In the presence of *p*-dimethylaminoazobenzene more riboflavin was retained in the liver when the fat of the diet was hydrogenated coconut oil than when it was corn oil. Hepatic tumors are known to form more rapidly when the latter oil is fed. In the presence of *m'*-methyl-*p*-dimethylaminoazobenzene, however, essentially the same amounts of riboflavin were found in the liver whether corn oil or hydrogenated coconut oil were fed, and on the basal diets used, the nature of the oil does not appear to affect the rate at which liver tumors develop when the *m'*-methyl dye is the carcinogen. These results, and the quantitative relationship between the carcinogenicity of the many azo dyes and their effects on hepatic riboflavin, suggest that riboflavin retention parallels the ability of the liver to resist the formation of tumors due to azo dyes.

THE LEVELS OF LIPIDS AND CARCINOGENIC AZO-DYES IN THE LIVERS OF RATS FED VARIOUS DIETS CONTAINING *p*-DIMETHYLAMINOAZOBENZENE. RELATIONSHIP TO THE FORMATION OF HEPATOMAS. HERBERT SILVERSTONE, and ALBERT TANNENBAUM. (Department of Cancer Research, Michael Reese Hospital, Chicago, Ill.)

The hypothesis that diets affect the formation of azo-dye-induced hepatomas in rats through modifying the level of carcinogenic azo dyes in the liver has been studied. The possibility that carcinogenicity might be influenced by the liver lipid level was also considered. Groups of 24 rats were fed the following diets: (a) brown rice; (b) brown rice plus 15 per cent brewers' yeast; (c) a "synthetic" diet high in protein and fat; (d) a "synthetic" diet low in protein and high in fat; (e) a similar "synthetic" diet low in both fat and protein. Six hundredths per cent *p*-dimethylaminoazobenzene was incorporated into each of the diets for 4 months; the dye was then omitted and the diets continued until death of the animal or the termination of the experiment 2 months later. The same diets with azo dye were also fed to groups of 5 rats for 7 weeks, following which the animals were sacrificed and their livers analyzed for total lipids, free and total cholesterol, lipid phosphorus, and carcinogenic azo dyes (*p*-dimethylaminoazobenzene plus *p*-monomethylaminoazobenzene). The levels of azo dyes appeared to be positively associated with the formation of hepatomas. There was no evidence that either hepatoma formation or the concentration of carcinogenic azo dye in the liver are dependent on the level of liver lipids.

INFLUENCE OF THIOURACIL UPON THE CARCINOGENIC ACTION OF ACETYLAMINOFLUORENE. K. E. PASCHKIS, A. CANTAROW, and J. STASNEY. (Jefferson Medical College and Hospital, Philadelphia, Pa.)

Rats fed 2-acetylaminofluorene develop a variety of malignant tumors. We have reported previously that treatment with certain sex hormones hastens and intensifies the development of cancer of the liver by this carcinogen. We have now found that administration of thiouracil protects the liver against the carcinogenic and other effects of acetylaminofluorene and also against the "potentiated" carcinogenicity of combined acetylaminofluorene and testosterone treatment. At the same time there are indications that the androgenic effect of testosterone is more pronounced in animals receiving thiouracil than in those receiving the hormone alone. These findings suggest that the protective action of thiouracil may consist, in part at least, in preventing the transformation of testosterone to a compound of carcinogenic (or co-carcinogenic) and at the same time of lessened androgenic potency.

Thiouracil treatment failed to protect the liver against the carcinogenic effect of dimethylaminoazobenzene.

The thyroid glands of animals treated with acetylaminofluorene and thiouracil show essentiaily the same changes (hyperplasia, adenoma) as those observed in rats treated over long periods of time with thiouracil alone. Malignancy of the thyroid developed in a few animals treated with acetylaminofluorene and thiouracil. Inasmuch as this has been reported by others in rats treated with thiouracil alone, the carcinogen appears merely to hasten and intensify the development of thyroid malignancy without being essential to it.

THE CARCINOGENICITY OF CERTAIN COMPOUNDS RELATED TO *p*-DIMETHYLAMINOAZOBENZENE. KANEMATSU SUGIURA. (Memorial Hospital, New York, N. Y.)

Many aminoazobenzene derivatives have been tested for carcinogenic activity in the rat. *N*,*N*-dimethyl-*p*-aminoazobenzene and *N*-methyl-*p*-aminoazobenzene have been found to be equally carcinogenic. They produced cholangiomas and hepatomas in all animals tested in approximately the same period of time. *N*,*N*-dimethyl-3′-methyl-*p*-aminoazobenzene was more carcinogenic than the parent compound *N*,*N*-dimethyl-*p*-aminoazobenzene; but the *N*,*N*-dimethyl-2′-methyl-*p*-aminoazobenzene and *N*,*N*-dimethyl-4′-methyl-*p*-aminoazobenzene were very much less active. *N*,*N*-diethyl-*p*-aminoazobenzene and all other higher alkyl homologues of *N*,*N*-dimethyl-*p*-aminoazobenzene tested failed to produce cirrhosis or neoplastic changes in the liver of the rat when fed in equimolecular amounts.

The investigation has been extended to several compounds of this series which have not been previously tested. Rats were fed a rice diet to which 0.06 per cent of *N*,*N*-dimethyl-*p*-aminoazobenzene dissolved in cottonseed oil or molar equivalent amounts of the other compounds were added. The diet was supplemented with a slice of fresh carrot and water daily. Feeding was continued until the animals either succumbed or were sacrificed at the end of the experimental period of 250 days. The results showed the *N*-methyl-3′-methyl-*p*-aminoazobenzene was at least as carcinogenic as the *N*-methyl or *N*,*N*-dimethyl compound. However, the *N*-methyl-2′-methyl-*p*-aminoazobenzene and the *N*-methyl-4′-methyl-*p*-aminoazobenzene were very much less carcinogenic. Although *N*,*N*-diethyl-*p*-aminoazobenzene was noncarcinogenic, *N*-methyl-*N*-ethyl-*p*-aminoazobenzene was definitely carcinogenic, an indication of the importance of the methyl radical for carcinogenesis. *N*,*N*-diethanol-*p*-aminoazobenzene was also noncarcinogenic. The livers of rats fed *N*,*N*-dimethyl-4′-hydroxy-*p*-aminoazobenzene had smooth surfaces and histological examination showed no evidence of tumors, bile duct changes, or abnormal regeneration of the ducts and liver cells, or any abnormal nuclear alteration.

American Association for Cancer Research, Inc.

38th Annual Meeting

Hotel Stevens, Chicago, Illinois

May 16 and 17, 1947

Proceedings of Business Sessions

Minutes of the Meeting of the Board of Directors Held May 15, 1947

The Board of Directors met at 8:30 p.m., May 15, 1947 at the Stevens Hotel in Chicago, Illinois. Drs. Bittner, Brues, Cowdry, Doisy, Furth, Gardner, Huggins, Little, Shear and Taylor were present.

It was voted that the reading of the minutes of the last meeting be waived.

REPORTS OF OFFICERS

The Chairman of the Board exhibited charts showing the number of new members elected and the number of papers presented at the annual meetings of the Association since 1915. By vote of the Board these charts are reproduced here. The Chairman also showed a list of the Officers, Directors and Councillors of the Association since 1915.

The Acting Treasurer announced the receipt during the year of several gifts totalling $51.00, largely in memory of Mrs. Marcella Dill. After brief discussion, it was voted that these and other gifts to the Association be segregated as a special Journal Fund.

The Treasurer's report was read and on the motion of Dr. M. J. Shear, who had been appointed auditor, was accepted.

REPORTS OF COMMITTEES

Program Committee.—Chairman J. J. Bittner reported that all of the papers submitted for presentation were accepted and placed on the program.

Nominating Committee.—Chairman H. C. Taylor, Jr. reported that his Committee had nominated for members of the Board of Directors to serve until 1950: Drs. A. M. Brues, B. L. Coley, E. T. Engle, J. Furth, C. D. Haagensen, J. G. Kidd, C. C. Little and Shields Warren. These names were listed on the proxies sent to the members of the Association by the Acting Secretary. Count of the proxies showed that Drs. Brues, Furth, Little and Warren received the largest number of votes. It was voted, "That the Acting-Secretary cast one vote for the nominees chosen by the members." The new Directors were then declared elected.

Membership Committee.—Chairman Charles Huggins reported that the Association now had 517 active members and 8 emeritus or honorary members.

The nominations for active membership were presented. Fifty-five candidates were recommended for election. They were:

Albright, Fuller, M.D., Massachusetts General Hospital, Boston, Mass.

Baumberger, J. Percy, D.Sc., Stanford University, California.

Beck, Lyle V., Ph.D., Hahnemann Medical College, Philadelphia, Pa.

Begg, Robert William, M.D., Dalhousie University, Halifax, N. S., Canada.

Bennett, Warren A., M.D., Mayo Clinic, Rochester, Minn.

Bierman, Howard, R., M.D., University of California Hospital, San Francisco, Calif.

Bowman, Russel O., Ph.D., Rhode Island Hospital, Providence, R. I.

Burdette, Walter J., Ph.D., M.D., Louisiana State University School of Medicine, New Orleans, La.

Caceres, Eduardo, M.D., San Marcos University, Lima, Peru.

Curtis, Maynie R., Ph.D., Detroit Institute of Cancer Research, Detroit, Mich.

Cutting, Windsor C., M.D., Stanford University School of Medicine, San Francisco, Calif.

Diller, Irene Corey, Ph.D., The Institute for Cancer Research, Lankenau Hospital, Philadelphia, Pa.

Duboff, Gregory, M.S., 504 Franklin St., Buffalo, N. Y.

Dubnik, Celia S., B.A., National Cancer Institute, Bethesda, Md.

Dunning, Wilhelmina F., Ph.D., Wayne University College of Medicine, Detroit, Mich.

Engel, R. W., Ph.D., Alabama Polytechnic Institute, Auburn, Ala.

Fishback, Hamilton R., Sc.D., M.D., Northwestern University Medical School, Chicago, Ill.

Friedman, Nathan B., M.D., Army Institute of Pathology, Washington, D. C.

Garcia, German Garcia, M.D., Hospitol Espanol, Mexico, D. F.

Gessler, Albert E., Ph.D., Interchemical Corporation, New York, N. Y.

Goland, Philip P., M.D., University of Pennsylvania, Philadelphia, Pa.

Gordon, Myron, Ph.D., American Museum of Natural History, New York, N.Y.

Greene, Harry Jonathan, M.D., Kings County Hospital, Brooklyn, N. Y.

György, Paul, M.D., University of Pennsylvania School of Medicine, Philadelphia, Pa.

Ham, Arthur Worth, M.D., University of Toronto, Toronto, Ontario, Canada.

Hauschka, Theodore Spaeth, Ph.D., The Institute for Cancer Research, Lankenau Hospital, Philadelphia, Pa.

Hollander, Franklin, Ph.D., The Mount Sinai Hospital, New York, N. Y.

Hummel, Katherine Pattee, Ph.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

Jones, Howard W., Jr., M.D., Johns Hopkins University, Baltimore, Md.

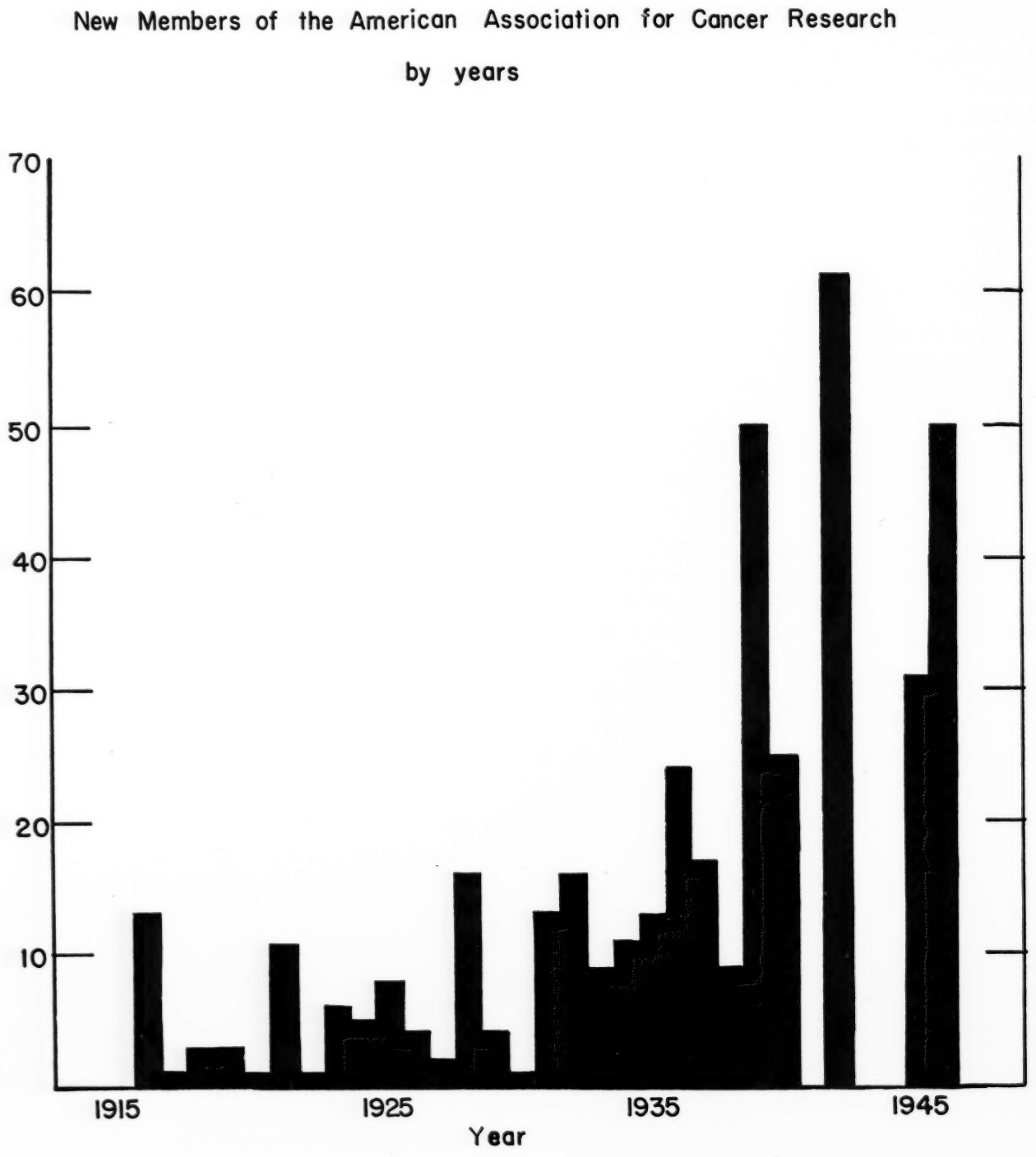
New Members of the American Association for Cancer Research
by years
70
60
50
40
30
20
10
1915
1925
1935
1945
Year

FIG. 1

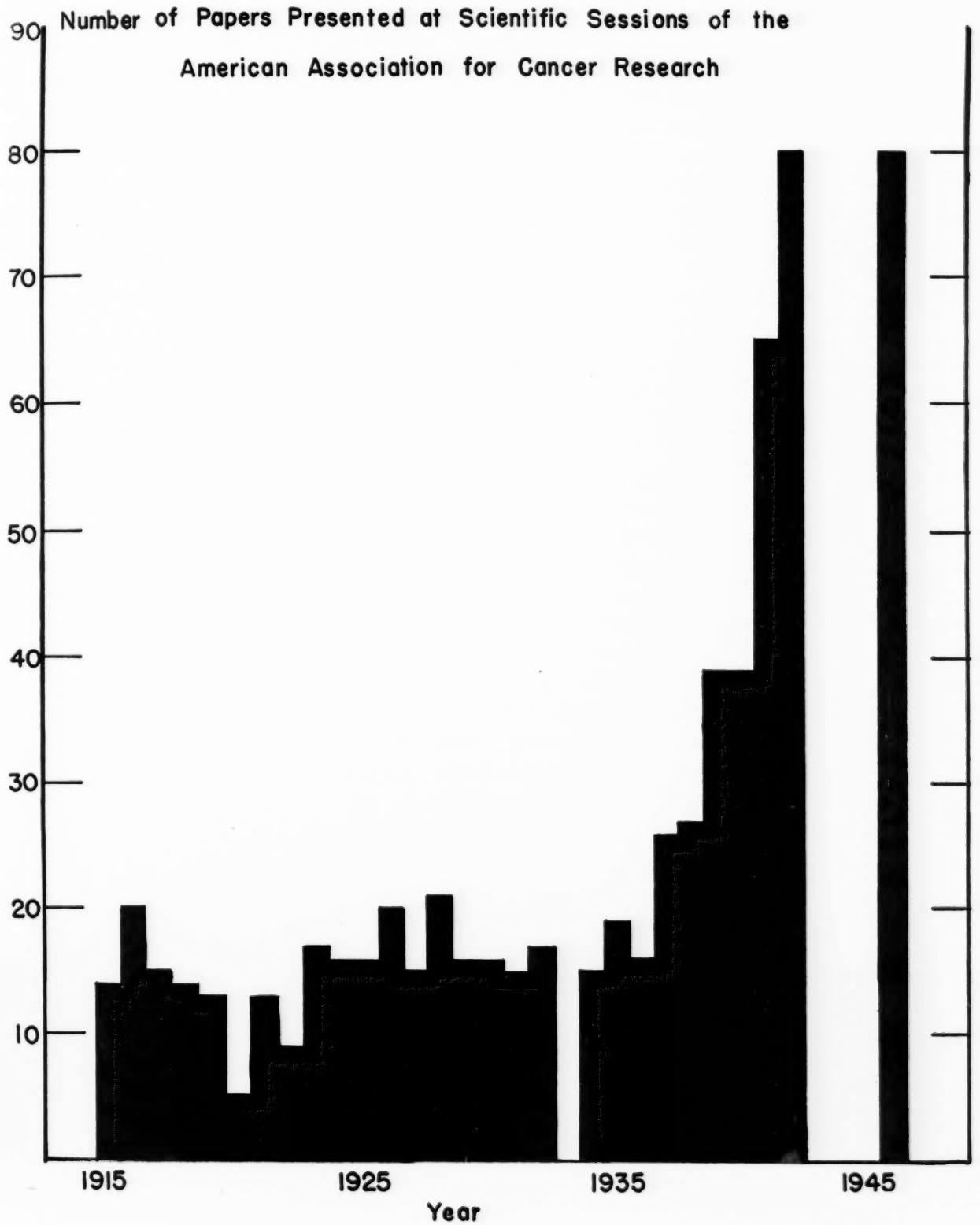
Number of Papers Presented at Scientific Sessions of the American Association for Cancer Research
90
80
70
60
50
40
30
20
10
1915
1925
1935
1945
Year

FIG. 2

April 30, 1947

Cash on deposit (Union & New Haven Trust Co.) March 1, 1946			$2441.96
Cash on hand, March 1, 1946			67.00
			2508.96
Checks outstanding, March 1, 1946			20.03
			2488.93
Cash on deposit (Greenwich Savings Bank) March 1, 1946			635.10
Interest on Savings Account to January 1, 1947			9.55
Receipts March 1, 1946 to April 30, 1947			
Dues collected			1607.66
Gifts			51.00
Subscriptions to *Cancer Research*			20.00
			4812.24
Disbursements March 1, 1946 to April 30, 1947			
Secretarial Assistance		$ 150.00	
Printing (Nomination forms, billheads, letterheads and ballots.)		64.00	
Stamps		79.15	
Reprints of By-Laws and list of members	$80.63		
Envelopes and labels for mailing	20.38	101.01	
Meeting March 11 and 12, 1946		287.14	
Registration cards, identification badges and tickets	46.25		
Rental on projector	10.00		
Programs	95.00		
Stenographic service	38.36		
Annual dinner (City tax, gratuities)	88.63		
Expenses of Program Committee	8.90		
Bank charges on foreign checks		1.69	
Subscriptions to *Cancer Research*		20.00	
Assessment for *Cancer Research*			
1946		475.00	
1947		522.00	
Fourth International Cancer Research Congress		512.63	
Contribution	500.00		
Cable and telephone	12.63		
National Society for Medical Research		50.00	
Refund on overpaid dues		6.00	
		$2269.12	$4812.24
Balance April 30, 1947			2543.12
Dues receivable			488.00
			$3031.12

CHARLES W. HOOKER, *Acting Secretary-Treasurer*

I hereby certify that the accounts and vouchers in the American Association for Cancer Research, Inc., for the above recorded period have been examined by me, and that the above are true statements of its financial operations and of its financial conditions as of April 30, 1947.

M. J. SHEAR, *Auditor for the Directors*

KAHN, REUBEN L., D.Sc., University Hospital, University of Michigan, Ann Arbor, Mich.

LASZLO, DANIEL, M.D., Montefiore Hospital, New York, N. Y.

LEBLOND, CHARLES PHILIPPE, M.D., PH.D., McGill University Medical School, Montreal, Quebec, Canada.

LISCO, HERMANN, M.D., Argonne National Laboratory, Chicago, Ill.

MCCUTCHEON, MORTON, M.D., University of Pennsylvania Medical School, Philadelphia, Pa.

MEYER, LEO MARTIN, M.D., 550 East 16th St. Brooklyn, New York.

MILLER, FRANKLIN R., M.D., Jefferson Medical College and Hospital, Philadelphia, Pa.

MONTGOMERY, HUGH, M.D., Hospital of the University of Pennsylvania, Philadelphia, Pa.

MOREHEAD, ROBERT PAGE, M.D., The Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

NIGRELLI, ROSS F., PH.D., New York Zoological Society, New York, N. Y.

OWEN, PHILIP S., M.D., National Research Council, Washington, D. C.

PARKER, RAYMOND C., PH.D., University of Toronto, Toronto, Ontario, Canada.

POOL, JOHN LAWRENCE, M.D., 140 East 154th street, New York, N. Y.

QUASTLER, HENRY, M.D., Carle Hospital Clinic, Urbana, Ill.

REIFENSTEIN, EDWARD C., JR., M.D., Massachusetts General Hospital, Boston, Mass.

ROBERTS, EUGENE, PH.D., Barnard Free Skin and Cancer Hospital, St. Louis, Mo.
ROBERTSON, WM. V. B., PH.D., College of Medicine, University of Vermont, Burlington, Vt.
RUNNER, MEREDITH N., PH.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.
ROYSTER, HENRY P., M.D., University of Pennsylvania School of Medicine, Philadelphia, Pa.
SALOMON, KURT, M.D., PH.D., University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.
SCARBOROUGH, J. ELLIOT, M.D., Emory University Hospital, Emory University, Ga.
SELIGMAN, ARNOLD M., M.D., Beth Israel Hospital and Harvard Medical School, Boston, Mass.
SMITH, PAUL KENNETH, PH.D., George Washington University School of Medicine, Washington, D. C.
STASNEY, JOSEPH, M.D., Jefferson Medical College, Philadelphia, Pa.
STERN, KURT, M.D., Mount Sinai Hospital, Chicago, Ill.
WHITE, PHILIP R., PH.D., The Institute for Cancer Research, Lankenau Hospital, Philadelphia, Pa.

It was recommended that two nominees be elected contributing members:

CARL A. GERSTACKER, B.S.E., Elsa U. Pardee Foundation, Midland, Mich.
ROBERT P. MEADER, M.D., 4002 Jenkins Arcade, Pittsburgh, Pa.

It was recommended that the following distinguished scientists be elected Honorary Members:

DR. E. C. DODDS, London
DR. E. L. KENNAWAY, London
DR. A. LACASSAGNE, Paris

It was voted "That the report of the Committee be accepted and their recommendations be adopted." The nominees were then declared elected as recommended.

Dr. Huggins then presented the recommendation of his Committee with respect to the *qualifications for membership* as specified in the By-Laws. As amended, the recommendation reads:

"The Committee recommends the following changes in the By Laws of the Association:

1. That Section 1 and 2 of Article I of the By-Laws be rescinded.

2. That the following clause be substituted for Section I of Article I:

Section 1, Members.

(a) The Association is to consist of three classes: active members, emeritus members and honorary members.

(b) Candidates for active membership shall be workers in good professional standing who have been active for at least two years in cancer research or for three years in research with one year in cancer research. Any person with these qualifications who has conducted and published meritorious researches in the field of cancer research and who is a resident of the Americas shall be eligible for active membership in the Association.

(c) Emeritus members are those who have attained the age of 65 years and have been members for ten years or more. They shall be exempt from dues.

(d) Distinguished scientists and others who have contributed to the advance of cancer research shall be eligible for election as honorary members of the Association. Honorary members shall be exempt from dues; they shall have the right to attend the meetings of the Association and of taking part in its scientific discussions, but they shall have no vote.

3. That Section 2 of Article I be amended to read:

Section 2. Election of Members.—The Board of Directors at any time and from time to time may elect to membership persons who meet the qualifications listed in Section 1.

4. That the changes in the rules regarding membership be made not retroactive from May 1947."

In the discussion of the proposal it was pointed out that under Section 1, (c) the transfer to Emeritus Membership becomes automatic and does not necessitate application by the member as heretofore.

It was voted "That the recommended changes in the By-Laws be approved and adopted." It was also voted "That the Membership Committee be given a hearty vote of thanks for a splendid job."

The Acting Secretary reported that applications for transfer to Emeritus Membership had been received from Dr. W. F. Jacobs and Dr. E. B. Krumbhaar. The transfers were approved.

The resignations of four members were accepted:

ALVIN J. COX, San Francisco, California
CARL F. SIEKMAN, Buffalo, New York
LESTER F. WICKS, St. Louis, Missouri
MELVIN C. REINHARD, Buffalo, New York

The deaths of the following members were regretfully announced.

HALSEY J. BAGG
STARLING W. CHILDS
ARTHUR E. HERTZLER
BARNET JOSEPH
WARD J. MACNEAL
GEORGE H. SEMKEN
BURTON T. SIMPSON
ROLLIN H. STEVENS

Journal Committee.—Chairman M. J. Shear reported that his Committee met in Philadelphia on February 13, 1947. The approximate cost of publishing *Cancer Research* in 1946 was $16,500. For the same year there were 895 subscriptions. Approximately 230 subscriptions were at $5.00 (yielding $1,150) and 660 at $7.00 (yielding $4,620); the total received from subscriptions was thus $5,770. The Association contributes to the journal $1.00 of the $3.00 dues paid by the 530 members. The total income was, then, about $6,300, leaving an apparent deficit of about $10,000.

The deficit has been met by contributions from the Donner Foundation, The Jane Coffin Childs Fund, and the Anna Fuller Fund. The Pardee Fund has asked to participate, and W. W. Allen of that Fund has been appointed to the Advisory Board of *Cancer Research*.

The Journal Committee has raised the following questions:

(1) Would it be advisable to raise the dues from $3 to $7, giving the journal $5 instead of $1, and giving each member, automatically, a paid subscription to *Cancer Research*? At present, members who subscribe are paying $8 instead of the proposed $7.

(2) Since only 182 of the 530 members subscribe, should an effort be made to stimulate subscriptions by members if the above question receives an adverse response?

(3) Should an effort be made to conduct a subscription drive among scientists, clinicians and others who might subscribe if the journal were called to their attention in an effective fashion?

The Committee viewed with approval the idea of attempting to establish an endowment for the journal. It also recommended that the Association encourage its members to submit more of their original papers to *Cancer Research*, and that it authorize the Editor to stimulate competent workers to prepare reviews on selected subjects for the journal.

Now that the subscription list is approaching 1,000, the Business Manager has been requested to investigate the possibility of securing advertisements.

The question given greatest attention was that of increasing the annual dues of the members and making subscriptions automatic. It was voted "That the proposal be referred to the members of the Association." It was also voted, with one Director dissenting, "That a favorable recommendation of the Board of Directors accompany the referral."

UNFINISHED BUSINESS

The Chairman reported the results of his inquiry into the possibility and desirability of the Association's affiliating with the Federation of American Societies for Experimental Biology. It was voted "That consideration of affiliation with the Federation be terminated."

It was disclosed that no financial report of the last International Cancer Research Congress is available.

The Chairman reviewed the desirability of having a history of the Association prepared and reported that Dr. W. H. Woglom declined appointment as historian. It was voted "That the Chairman appoint a Committee to obtain from the older members of the Association data and their recollections on the early history of the Association." This action was regarded necessary in view of the lack of data from 1907, the year of organization, to 1915. Dr. C. C. Little was appointed Chairman of the committee and instructed to select his own associates.

NEW BUSINESS

The World Health Organization and the desirability of the Association's participation were discussed briefly. It was decided that the next Chairman of the Board appoint a committee to formulate recommendations.

Nominations for officers for the coming year were then made; For President, John J. Bittner; for Vice-President, Charles Huggins. It was voted "That Charles W. Hooker be appointed Secretary-Treasurer for the coming year and be made ex-officio a member of the Board of Directors."

It was voted that the costs of conducting the annual meeting be paid from the funds of the Association.

The meeting was adjourned at 12:00 midnight.

WILLIAM U. GARDNER,
Chairman, Board of Directors
CHARLES W. HOOKER,
Acting Secretary

MINUTES OF THE MEETING OF THE MEMBERS HELD MAY 16, 1947

The meeting of the members of the Association was called to order at 1:45 p.m., May 16, 1947 at the Stevens Hotel in Chicago, Illinois.

Reading of the minutes of the last meeting was omitted on vote of the members.

The reports of the Acting Treasurer and Auditor were presented and approved.

The results of the count of proxy votes for new Directors to serve until 1950 and the action of the Board of Directors were reported as recorded in the minutes of the meeting of the Board.

The nominations for officers of the Association made by the Board of Directors were read:

JOHN J. BITTNER, *President*
CHARLES HUGGINS, *Vice President*
CHARLES W. HOOKER, *Secretary-Treasurer*

The candidates were considered separately and elected.

The list of newly elected members was read by the Secretary. These names are recorded in the minutes of the meeting of the Board of Directors on May 15, 1947.

The change in the By-Laws concerning qualifications for membership adopted by the Board of Directors was read. It was voted "That the change be approved."

The proposal that the annual dues of members be increased from $3.00 to $7.00 and include a subscription to *Cancer Research* was presented and discussed. It was voted "That the desire of the members be ascertained by distribution of printed ballots by mail."

The meeting adjourned at 2:31 p.m.

WILLIAM U. GARDNER,
President
CHARLES W. HOOKER,
Secretary

MINUTES OF THE MEETING OF THE BOARD OF DIRECTORS HELD MAY 17, 1947

The meeting was called to order at 12:42 p.m. at the Stevens Hotel in Chicago, Illinois, following waiver of previous formal notice of the meeting of the Directors signed by all Directors present and constituting a quorum. Present were Directors Aub, Bittner, Brues, Cowdry, Doisy, Gardner, Huggins and Shear.

The question of publication of abstracts prior to the annual meeting was again discussed. It was voted "That the present procedure be continued next year and that the Program Committee submit recommendations as to the make-up and handling of the program." The question of symposia being held in conjunction with the annual meeting was also discussed and it was voted "That consideration of symposia be made the responsibility of the Program Committee for decision and action."

Dr. W. U. Gardner was appointed chairman of a committee to investigate the World Health Organization.

Dr. C. C. Little was appointed chairman of a committee on memorials for deceased members.

It was voted "That Chairman appoint a member of the Association to examine the desirability of affiliating with the Union of Biological Societies and submit a report."

NEW BUSINESS

It was unanimously resolved, "That Charles W. Hooker, Secretary-Treasurer and John J. Bittner, President, be, and each of them hereby is authorized in the name and on behalf of the Corporation to open a bank account or bank accounts with such banks, bankers and/or trust companies as they or each of them shall determine, and to deposit therein to the credit of the Corporation from time to time any and all monies and checks of the Corporation; and

Resolved, that the banks, bankers, and/or trust companies so designated as depositaries of the Corporation be, and they hereby are, severally authorized to honor and pay all checks, drafts, and other orders for the payment of money drawn upon such account or accounts (including checks, drafts, or other orders of one or both of the persons making, signing, or drawing them) made, signed or drawn by the following persons: Charles W. Hooker or John J. Bittner."

It was also "*Resolved*, that the Greenwich Savings Bank of 1356 Broadway and 985 Sixth Avenue, Borough of Manhattan, City of New York, is hereby designated as depositary of funds of this corporation and is authorized to honor drafts and orders for the payment and withdrawal of moneys therefrom made in the name of this corporation and signed by President, John J. Bittner, or Secretary-Treasurer Charles W. Hooker.

"And it is Further *Resolved* that the foregoing authority shall continue until written notice of revocation of this Resolution shall be received by the Greenwich Savings Bank.

"And it is Further *Resolved* that said The Greenwich Savings Bank is authorized to accept the certificate of the Secretary of this corporation as evidence of the names and signatures of the persons at any time authorized to act pursuant to this Resolution."

It was voted "That the sum $250 be allocated for secretarial assistance to be used as necessary."

It was voted "That the publication of the minutes and the scientific proceedings of the meeting be authorized and that the cost of publication be paid by the Association."

The Chairman of the Board proposed the following standing committees:

Program Committee.—J. C. AUB, *Chairman:* A. M. BRUES, E. A. DOISY.

Nominating Committee.—W. U. GARDNER, *Chairman:* W. E. HESTON, ALBERT TANNENBAUM.

Membership Committee.—CHARLES HUGGINS, *Chairman:* A. M. BRUES, J. FURTH.

Journal Committee.—M. J. SHEAR, *Chairman:* S. BAYNE-JONES, M. W. S. SCHRAM.

Cancer Research, its Organization and Support.—SHIELDS WARREN, *Chairman:* CHARLES HUGGINS, G. M. SMITH.

The Board approved the proposed Committees.

It was voted "That the report of the *Committee on Cancer Research, its Organization and Support* be published as part of the minutes of the meeting." The report, which was read at the annual dinner, follows:

"*The Committee on Cancer Research, its Organization and Support*, felt that its first responsibility was to determine the attitude of those carrying on cancer research, the members of this Association, toward the Association taking a more active part in such matters and to determine their views with regard to forms of support for cancer research recently or currently planned.

"The questionnaire sent to the members is reproduced herewith and the replies tabulated. Many members sent additional comments, which have been used to help formulate additional recommendations.

"To summarize the viewpoint of the membership as a whole as presented by the questionnaire, there is a predominant feeling that the Association should actively interest itself in recommendations for the financial support of cancer research and also a federal subsidy is desirable. However, a lump sum appropriation is opposed. It was felt that no federal funds for cancer research should be administered through state health departments. The majority of members felt the present method of disbursement of federal funds for cancer research and of funds for cancer research by the American Cancer Society through the Committee on Growth to be satisfactory. However, approximately one-third of those voting felt that the present methods were not entirely satisfactory.

"It is obvious that most members desire their Association to take an active part in the organization and support of cancer research.

"The formal organization of cancer research, or of any research, is of uncertain value. Only when fundamental principles are known, and objectives clearly defined in relation to this knowledge can organization be expected to yield fruitful results. Organization of research to prevent duplication, to guide development, tends to sterility and to the production of "pot-boilers." Such organization might well have prevented the discovery of insulin on the ground that the experiments had been done before.

"Cancer research has not yet reached and probably will never reach the stage where organization of a directional type can be other than hampering. Organization for the dissemination of knowledge in the field is desirable. Increased support should be given our official journal, *Cancer Research*. Conferences such as those sponsored by the American Cancer Society, the Donner Foundation and others, of those working in specialized fields are of value, as well as such general conferences as our projected Fourth International Cancer Research Congress next September.

"Support of Cancer Research should be continuing rather than on a lump sum basis with a short time limit, as in the proposed Pepper-Nealy Bill of the last session.

The problem of cancer is not so nearly solved that a time limit can be set for final success. The system of annual grants has been widely recognized as unsatisfactory, and most major grants-in-aid are now made with a tacit if not actual understanding that support will continue for several years.

"Two distinct types of research aimed toward the solution of the cancer problem exist—work on fundamental biological, chemical and physical problems that may shed light on abnormal growth and its control, and work directly aimed at understanding and controlling neoplastic growth. At the present the former type is receiving greater emphasis in part because many investigators believe cancer can be understood only through broad advances in biology, in part because of lack of data to permit a frontal attack, in part because some of those bringing new knowledge and new techniques to bear on the cancer problem have little or no knowledge of the disease.

"One of the most acute problems at present is to conserve for continued cancer research the young and able investigator as he matures. The present system of grants-in-aid provides adequately for investigators of fellowship grade, but when a worker of proved ability reaches his late thirties or forty, annual grants and salaries less than those of bus drivers or brick-layers are insufficient. A heavy loss of highly trained research workers is thus inevitable. Special effort should be made to provide means of encouraging these to remain in the cancer research field and to capitalize on their experience."

It was voted "That the next annual meeting be held in conjunction with the meeting of the Federation of American Societies for Experimental Biology, preceding that meeting if possible."

With respect to the program it was agreed that there be no change in the current practice of designating non-members as presenting papers "By invitation". It was also agreed that the Program Committee should make recommendations as to the style of abstracts acceptable.

Concern was expressed over the interruption of publication of *Cancer Research*. After discussion,

It was Resolved "That the Board of Directors of the American Association for Cancer Research appreciates the difficulties facing the journal, but wishes to emphasize the urgency of prompt resumption of publication in order that the accelerated research in cancer be made available to investigators."

The meeting was adjourned at 1:30 p.m.

JOHN J. BITTNER,
Chairman, Board of Directors

CHARLES W. HOOKER,
Secretary

RESULTS OF THE VOTING

	Yes	No	No vote
1. (a) Should the Association limit its activities to the publication and interchange of scientific information?	42	119	97
or			
(b) Should the Association also be interested in recommendations for the financial support of cancer research?	223	23	12
2. Do you favor a Federal subsidy for cancer research?	225	27	6
3. Is a Federal lump sum appropriation of $100,000,000 for cancer research the most satisfactory type of support?	101	107	50
4. Should the Association make recommendations for the administration of such an appropriation?	223	27	8
5. Should Federal funds for cancer research be made directly available to			
(a) Institutions for cancer research	219	18	21
(b) Universities	214	22	22
(c) Hospitals	183	40	35
(d) Individual investigators	185	49	24
6. Should Federal funds for cancer research be administered by state health departments?	21	231	6
7. Should the Association seek representation in any Federal agency established to distribute cancer research funds?	219	32	7
8. Is it your opinion that the Association should have representation in other national public agencies set up to collect funds from the public to distribute them for cancer research?	213	42	3
	Satisfactory	Unsatisfactory	No Opinion
9. Do you consider the method of disbursement of Federal funds for cancer research on recommendation of the National Advisory Cancer Council of the United States Public Health Service as satisfactory, unsatisfactory, or have you no opinion?	114	50	94
10. Do you consider the method of disbursement of funds for cancer research by the American Cancer Society through the Growth Committee of the National Research Council as satisfactory, unsatisfactory, or have you no opinion?	108	53	97